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(54) Title: METHOD FOR INTEGRATING GENES AT SPECIFIC SITES IN MAMMALIAN CELLS VIA HOMOLOGOUS RECOMBINATION AND VECTORS FOR ACCOMPLISHING THE SAME			
(57) Abstract A method for achieving site specific integration of a desired DNA at a target site in a mammalian cell via homologous recombination is described. This method provides for the reproducible selection of cell lines wherein a desired DNA is integrated at a predetermined transcriptionally active site previously marked with a marker plasmid. The method is particularly suitable for the production of mammalian cell lines which secrete mammalian proteins at high levels, in particular immunoglobulins. Vectors and vector combinations for use in the subject cloning method are also provided.			

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Title of the Invention

METHOD FOR INTEGRATING GENES AT SPECIFIC SITES IN MAMMALIAN CELLS VIA HOMOLOGOUS RECOMBINATION AND VECTORS FOR ACCOMPLISHING THE SAME

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Field of the Invention

The present invention relates to a process of targeting the integration of a desired exogenous DNA to a specific location within the genome of a mammalian cell. 10 More specifically, the invention describes a novel method for identifying a transcriptionally active target site ("hot spot") in the mammalian genome, and inserting a desired DNA at this site via homologous recombination. The invention also optionally provides the ability for 15 gene amplification of the desired DNA at this location by co-integrating an amplifiable selectable marker, e.g., DHFR, in combination with the exogenous DNA. The invention additionally describes the construction of novel vectors suitable for accomplishing the above, and 20 further provides mammalian cell lines produced by such methods which contain a desired exogenous DNA integrated at a target hot spot.

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Background

Technology for expressing recombinant proteins in both prokaryotic and eukaryotic organisms is well established. Mammalian cells offer significant advantages over bacteria or yeast for protein production, resulting from their ability to correctly assemble, glycosylate and post-translationally modify recombinantly expressed proteins. After transfection into the host cells, recombinant expression constructs can be maintained as extrachromosomal elements, or may be integrated into the host cell genome. Generation of stably transfected mammalian cell lines usually involves the latter; a DNA construct encoding a gene of interest along with a drug resistance gene (dominant selectable marker) is introduced into the host cell, and subsequent growth in the presence of the drug allows for the selection of cells that have successfully integrated the exogenous DNA. In many instances, the gene of interest is linked to a drug resistant selectable marker which can later be subjected to gene amplification. The gene encoding dihydrofolate reductase (DHFR) is most commonly used for this purpose. Growth of cells in the presence of methotrexate, a competitive inhibitor of DHFR, leads to increased DHFR production by means of amplification of the DHFR gene. As flanking regions of DNA will also become amplified, the resultant coamplification of a DHFR linked gene in the transfected cell line can lead to increased protein

production, thereby resulting in high level expression of the gene of interest.

While this approach has proven successful, there are a number of problems with the system because of the random nature of the integration event. These problems exist because expression levels are greatly influenced by the effects of the local genetic environment at the gene locus, a phenomena well documented in the literature and generally referred to as "position effects" (for example, see Al-Shawi et al, *Mol. Cell. Biol.*, 10:1192-1198 (1990); Yoshimura et al, *Mol. Cell. Biol.*, 7:1296-1299 (1987)). As the vast majority of mammalian DNA is in a transcriptionally inactive state, random integration methods offer no control over the transcriptional fate of the integrated DNA. Consequently, wide variations in the expression level of integrated genes can occur, depending on the site of integration. For example, integration of exogenous DNA into inactive, or transcriptionally "silent" regions of the genome will result in little or no expression. By contrast integration into a transcriptionally active site may result in high expression.

Therefore, when the goal of the work is to obtain a high level of gene expression, as is typically the desired outcome of genetic engineering methods, it is generally necessary to screen large numbers of transfec-tants to find such a high producing clone.

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Additionally, random integration of exogenous DNA into the genome can in some instances disrupt important cellular genes, resulting in an altered phenotype. These factors can make the generation of high expressing stable mammalian cell lines a complicated and laborious process.

Recently, our laboratory has described the use of DNA vectors containing translationally impaired dominant selectable markers in mammalian gene expression. (This is disclosed in U.S. Serial No. 08/147,696 filed November 3, 1993, recently allowed).

These vectors contain a translationally impaired neomycin phosphotransferase (neo) gene as the dominant selectable marker, artificially engineered to contain an intron into which a DHFR gene along with a gene or genes of interest is inserted. Use of these vectors as expression constructs has been found to significantly reduce the total number of drug resistant colonies produced, thereby facilitating the screening procedure in relation to conventional mammalian expression vectors.

Furthermore, a significant percentage of the clones obtained using this system are high expressing clones. These results are apparently attributable to the modifications made to the neo selectable marker. Due to the translational impairment of the neo gene, transfected cells will not produce enough neo protein to survive drug selection, thereby decreasing the overall

number of drug resistant colonies. Additionally, a higher percentage of the surviving clones will contain the expression vector integrated into sites in the genome where basal transcription levels are high, resulting in overproduction of neo, thereby allowing the cells to overcome the impairment of the neo gene. Concomitantly, the genes of interest linked to neo will be subject to similar elevated levels of transcription. This same advantage is also true as a result of the artificial intron created within neo; survival is dependent on the synthesis of a functional neo gene, which is in turn dependent on correct and efficient splicing of the neo introns. Moreover, these criteria are more likely to be met if the vector DNA has integrated into a region which is already highly transcriptionally active.

Following integration of the vector into a transcriptionally active region, gene amplification is performed by selection for the DHFR gene. Using this system, it has been possible to obtain clones selected using low levels of methotrexate (50nM), containing few (<10) copies of the vector which secrete high levels of protein (>55pg/cell/day). Furthermore, this can be achieved in a relatively short period of time. However, the success in amplification is variable. Some transcriptionally active sites cannot be amplified and

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therefore the frequency and extent of amplification from a particular site is not predictable.

Overall, the use of these translationally impaired vectors represents a significant improvement over other methods of random integration. However, as discussed, the problem of lack of control over the integration site remains a significant concern.

One approach to overcome the problems of random integration is by means of gene targeting, whereby the exogenous DNA is directed to a specific locus within the host genome. The exogenous DNA is inserted by means of homologous recombination occurring between sequences of DNA in the expression vector and the corresponding homologous sequence in the genome. However, while this type of recombination occurs at a high frequency naturally in yeast and other fungal organisms, in higher eukaryotic organisms it is an extremely rare event. In mammalian cells, the frequency of homologous versus non-homologous (random integration) recombination is reported to range from 1/100 to 1/5000 (for example, see Capecchi, *Science*, 244:1288-1292 (1989); Morrow and Kucherlapati, *Curr. Op. Biotech.*, 4:577-582 (1993)).

One of the earliest reports describing homologous recombination in mammalian cells comprised an artificial system created in mouse fibroblasts (Thomas et al, *Cell*, 44:419-428 (1986)). A cell line containing a mutated, non-functional version of the neo gene integrated into

the host genome was created, and subsequently targeted with a second non-functional copy of neo containing a different mutation. Reconstruction of a functional neo gene could occur only by gene targeting. Homologous 5 recombinants were identified by selecting for G418 resistant cells, and confirmed by analysis of genomic DNA isolated from the resistant clones.

Recently, the use of homologous recombination to replace the heavy and light immunoglobulin genes at 10 endogenous loci in antibody secreting cells has been reported. (U.S. Patent No. 5,202,238, Fell et al, 1993.) However, this particular approach is not widely applicable, because it is limited to the production of immunoglobulins in cells which 15 endogenously express immunoglobulins, e.g., B cells and myeloma cells. Also, expression is limited to single copy gene levels because co-amplification after homologous recombination is not included. The method is further complicated by the fact that two separate 20 integration events are required to produce a functional immunoglobulin: one for the light chain gene followed by one for the heavy chain gene.

An additional example of this type of system has 25 been reported in NS/0 cells, where recombinant immunoglobulins are expressed by homologous recombination into the immunoglobulin gamma 2A locus (Hollis et al, international patent application #

PCT/IB95 (00014).) Expression levels obtained from this site were extremely high - on the order of 20pg/cell/day from a single copy integrant. However, as in the above example, expression is limited to this level because an amplifiable gene is not cointegrated in this system.

Also, other researchers have reported aberrant glycosylation of recombinant proteins expressed in NS/0 cells (for example, see Flesher et al, *Biotech. and Bioeng.*, 48:399-407 (1995)), thereby limiting the applicability of this approach.

The cre-loxP recombination system from bacteriophage P1 has recently been adapted and used as a means of gene targeting in eukaryotic cells. Specifically, the site specific integration of exogenous DNA into the Chinese hamster ovary (CHO) cell genome using cre recombinase and a series of lox containing vectors have been described. (Fukushige and Sauer, *Proc. Natl. Acad. Sci. USA*, 89:7905-7909 (1992).) This system is attractive in that it provides for reproducible expression at the same chromosomal location. However, no effort was made to identify a chromosomal site from which gene expression is optimal, and as in the above example, expression is limited to single copy levels in this system. Also, it is complicated by the fact that one needs to provide for expression of a functional recombinase enzyme in the mammalian cell.

The use of homologous recombination between an introduced DNA sequence and its endogenous chromosomal locus has also been reported to provide a useful means of genetic manipulation in mammalian cells, as well as 5 in yeast cells. (See e.g., Bradley et al, *Meth. Enzymol.*, 223:855-879 (1993); Capecchi, *Science*, 244:1288-1292 (1989); Rothstein et al, *Meth. Enzymol.*, 194:281-301 (1991)). To date, most mammalian gene targeting studies have been directed toward gene 10 disruption ("knockout") or site-specific mutagenesis of selected target gene loci in mouse embryonic stem (ES) cells. The creation of these "knockout" mouse models has enabled scientists to examine specific structure-function issues and examine the biological 15 importance of a myriad of mouse genes. This field of research also has important implications in terms of potential gene therapy applications.

Also, vectors have recently been reported by Cell-tech (Kent, U.K.) which purportedly are targeted to 20 transcriptionally active sites in NSO cells, which do not require gene amplification (Peakman et al, *Hum. Antibod. Hybridomas*, 5:65-74 (1994)). However, levels of immunoglobulin secretion in these unamplified cells have not been reported to exceed 20pg/cell/day, while in 25 amplified CHO cells, levels as high as 100pg/cell/day can be obtained (Id.).

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It would be highly desirable to develop a gene targeting system which reproducibly provided for the integration of exogenous DNA into a predetermined site in the genome known to be transcriptionally active.

5 Also, it would be desirable if such a gene targeting system would further facilitate co-amplification of the inserted DNA after integration. The design of such a system would allow for the reproducible and high level expression of any cloned gene of interest in a mammalian
10 cell, and undoubtedly would be of significant interest to many researchers.

In this application, we provide a novel mammalian expression system, based on homologous recombination occurring between two artificial substrates contained in
15 two different vectors. Specifically, this system uses a combination of two novel mammalian expression vectors, referred to as a "marking" vector and a "targeting" vector.

Essentially, the marking vector enables the identification and marking of a site in the mammalian genome which is transcriptionally active, i.e., a site at which
20 gene expression levels are high. This site can be regarded as a "hot spot" in the genome. After integration of the marking vector, the subject expression system
25 enables another DNA to be integrated at this site, i.e., the targeting vector, by means of homologous recombination occurring between DNA sequences common to

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both vectors. This system affords significant advantages over other homologous recombination systems.

Unlike most other homologous systems employed in mammalian cells, this system exhibits no background.

5 Therefore, cells which have only undergone random integration of the vector do not survive the selection.

Thus, any gene of interest cloned into the targeting plasmid is expressed at high levels from the marked hot spot. Accordingly, the subject method of gene expres-

10 sion substantially or completely eliminates the problems inherent to systems of random integration, discussed in detail above. Moreover, this system provides reproduc-
ible and high level expression of any recombinant pro-
tein at the same transcriptionally active site in the

15 mammalian genome. In addition, gene amplification may be effected at this particular transcriptionally active site by including an amplifiable dominant selectable marker (e.g. DHFR) as part of the marking vector.

Objects of the Invention

20 Thus, it is an object of the invention to provide an improved method for targeting a desired DNA to a specific site in a mammalian cell.

It is a more specific object of the invention to provide a novel method for targeting a desired DNA to a specific site in a mammalian cell via homologous recom-
25 bination.

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It is another specific object of the invention to provide novel vectors for achieving site specific integration of a desired DNA in a mammalian cell.

5 It is still another object of the invention to provide novel mammalian cell lines which contain a desired DNA integrated at a predetermined site which provides for high expression.

10 It is a more specific object of the invention to provide a novel method for achieving site specific integration of a desired DNA in a Chinese hamster ovary (CHO) cell.

15 It is another more specific object of the invention to provide a novel method for integrating immunoglobulin genes, or any other genes, in mammalian cells at predetermined chromosomal sites that provide for high expression.

20 It is another specific object of the invention to provide novel vectors and vector combinations suitable for integrating immunoglobulin genes into mammalian cells at predetermined sites that provide for high expression.

25 It is another object of the invention to provide mammalian cell lines which contain immunoglobulin genes integrated at predetermined sites that provide for high expression.

It is an even more specific object of the invention to provide a novel method for integrating immunoglobulin

genes into CHO cells that provide for high expression, as well as novel vectors and vector combinations that provide for such integration of immunoglobulin genes into CHO cells.

5 In addition, it is a specific object of the invention to provide novel CHO cell lines which contain immunoglobulin genes integrated at predetermined sites that provide for high expression, and have been amplified by methotrexate selection to secrete even greater amounts
10 of functional immunoglobulins.

Brief Description of the Figures

Figure 1 depicts a map of a marking plasmid according to the invention referred to as Desmond. The plasmid is shown in circular form (1a) as well as a
15 linearized version used for transfection (1b).

Figure 2(a) shows a map of a targeting plasmid referred to "Molly". Molly is shown here encoding the anti-CD20 immunoglobulin genes, expression of which is described in Example 1.

20 Figure 2(b) shows a linearized version of Molly, after digestion with the restriction enzymes *Kpn*1 and *Pac*1. This linearized form was used for transfection.

Figure 3 depicts the potential alignment between Desmond sequences integrated into the CHO genome, and
25 incoming targeting Molly sequences. One potential ar-

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rangement of Molly integrated into Desmond after homologous recombination is also presented.

Figure 4 shows a Southern analysis of single copy Desmond clones. Samples are as follows:

- 5 Lane 1: λ HindIII DNA size marker
- Lane 2: Desmond clone 10F3
- Lane 3: Desmond clone 10C12
- Lane 4: Desmond clone 15C9
- Lane 5: Desmond clone 14B5
- 10 Lane 6: Desmond clone 9B2

Figure 5 shows a Northern analysis of single copy Desmond clones. Samples are as follows: Panel A: northern probed with CAD and DHFR probes, as indicated on the figure. Panel B: duplicate northern, probed with 15 CAD and HisD probes, as indicated. The RNA samples loaded in panels A and B are as follows:

- Lane 1: clone 9B2, lane 2; clone 10C12, lane 3; clone 14B5, lane 4; clone 15C9, lane 5; control RNA from CHO transfected with a HisD and DHFR containing plasmid,
- 20 lane 6; untransfected CHO.

Figure 6 shows a Southern analysis of clones resulting from the homologous integration of Molly into Desmond. Samples are as follows:

- Lane 1: λ HindIII DNA size markers, Lane 2: 20F4, lane 3; 25
- 5F9, lane 4; 21C7, lane 5; 24G2, lane 6; 25E1, lane 7; 28C9, lane 8; 29F9, lane 9; 39G11, lane 10; 42F9, lane 11; 50G10, lane 12; Molly plasmid DNA, linearized with

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BglII (top band) and cut with BglII and KpnI (lower band), lane 13; untransfected Desmond.

Figures 7A through 7G contain the Sequence Listing for Desmond.

5 Figures 8A through 8I contain the Sequence Listing for Molly-containing anti-CD20.

Figure 9 contains a map of the targeting plasmid, "Mandy," shown here encoding anti-CD23 genes, the expression of which is disclosed in Example 5.

10 Figures 10A through 10N contain the sequence listing of "Mandy" containing the anti-CD23 genes as disclosed in Example 5.

Detailed Description of the Invention

The invention provides a novel method for integrating a desired exogenous DNA at a target site within the genome of a mammalian cell via homologous recombination. 15 Also, the invention provides novel vectors for achieving the site specific integration of a DNA at a target site in the genome of a mammalian cell.

20 More specifically, the subject cloning method provides for site specific integration of a desired DNA in a mammalian cell by transfection of such cell with a "marker plasmid" which contains a unique sequence that is foreign to the mammalian cell genome and which 25 provides a substrate for homologous recombination, followed by transfection with a "target plasmid" containing

a sequence which provides for homologous recombination with the unique sequence contained in the marker plasmid, and further comprising a desired DNA that is to be integrated into the mammalian cell. Typically, the 5 integrated DNA will encode a protein of interest, such as an immunoglobulin or other secreted mammalian glycoprotein.

The exemplified homologous recombination system uses the neomycin phosphotransferase gene as a dominant 10 selectable marker. This particular marker was utilized based on the following previously published observations;

(i) the demonstrated ability to target and restore function to a mutated version of the neo gene (cited 15 earlier) and

(ii) our development of translationally impaired expression vectors, in which the neo gene has been artificially created as two exons with a gene of interest inserted in the intervening intron; neo exons are correctly spliced and translated *in vivo*, producing a functional protein and thereby conferring G418 resistance on the resultant cell population. In this application, the neo gene is split into three exons. The third exon of neo is present on the "marker" plasmid and becomes integrated into the host cell genome upon integration of the marker plasmid into the mammalian cells. Exons 1 and 2 are present on the targeting plasmid, and are separated 20 25

by an intervening intron into which at least one gene of interest is cloned. Homologous recombination of the targeting vector with the integrated marking vector results in correct splicing of all three exons of the neo gene and thereby expression of a functional neo protein (as determined by selection for G418 resistant colonies). Prior to designing the current expression system, we had experimentally tested the functionality of such a triply spliced neo construct in mammalian cells. The results of this control experiment indicated that all three neo exons were properly spliced and therefore suggested the feasibility of the subject invention.

However, while the present invention is exemplified using the neo gene, and more specifically a triple split neo gene, the general methodology should be efficacious with other dominant selectable markers.

As discussed in greater detail *infra*, the present invention affords numerous advantages to conventional gene expression methods, including both random integration and gene targeting methods. Specifically, the subject invention provides a method which reproducibly allows for site-specific integration of a desired DNA into a transcriptionally active domain of a mammalian cell. Moreover, because the subject method introduces an artificial region of "homology" which acts as a unique substrate for homologous recombination and the

insertion of a desired DNA, the efficacy of subject invention does not require that the cell endogenously contain or express a specific DNA. Thus, the method is generically applicable to all mammalian cells, and can
5 be used to express any type of recombinant protein.

The use of a triply spliced selectable marker, e.g., the exemplified triply spliced neo construct, guarantees that all G418 resistant colonies produced will arise from a homologous recombination event (random 10 integrants will not produce a functional neo gene and consequently will not survive G418 selection). Thus, the subject invention makes it easy to screen for the desired homologous event. Furthermore, the frequency of additional random integrations in a cell that has undergone a homologous recombination event appears to be low.
15

Based on the foregoing, it is apparent that a significant advantage of the invention is that it substantially reduces the number of colonies that need be screened to identify high producer clones, i.e., cell 20 lines containing a desired DNA which secrete the corresponding protein at high levels. On average, clones containing integrated desired DNA may be identified by screening about 5 to 20 colonies (compared to several thousand which must be screened when using standard 25 random integration techniques, or several hundred using the previously described intronic insertion vectors) Additionally, as the site of integration was preselected

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and comprises a transcriptionally active domain, all exogenous DNA expressed at this site should produce comparable, i.e. high levels of the protein of interest.

Moreover, the subject invention is further advantageous in that it enables an amplifiable gene to be inserted on integration of the marking vector. Thus, when a desired gene is targeted to this site via homologous recombination, the subject invention allows for expression of the gene to be further enhanced by gene amplification. In this regard, it has been reported in from the literature that different genomic sites have different capacities for gene amplification (Meinkoth et al, *Mol. Cell Biol.*, 7:1415-1424 (1987)). Therefore, this technique is further advantageous as it allows for the placement of a desired gene of interest at a specific site that is both transcriptionally active and easily amplified. Therefore, this should significantly reduce the amount of time required to isolate such high producers.

Specifically, while conventional methods for the construction of high expressing mammalian cell lines can take 6 to 9 months, the present invention allows for such clones to be isolated on average after only about 3-6 months. This is due to the fact that conventionally isolated clones typically must be subjected to at least three rounds of drug resistant gene amplification in order to reach satisfactory levels of gene expression.

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As the homologously produced clones are generated from a preselected site which is a high expression site, fewer rounds of amplification should be required before reaching a satisfactory level of production.

5 Still further, the subject invention enables the reproducible selection of high producer clones wherein the vector is integrated at low copy number, typically single copy. This is advantageous as it enhances the stability of the clones and avoids other potential adverse side-effects associated with high copy number. As described *supra*, the subject homologous recombination system uses the combination of a "marker plasmid" and a "targeting plasmid" which are described in more detail below.

10

15 The "marker plasmid" which is used to mark and identify a transcriptionally hot spot will comprise at least the following sequences:

(i) a region of DNA that is heterologous or unique to the genome of the mammalian cell, which functions as
20 a source of homology, allows for homologous recombination (with a DNA contained in a second target plasmid). More specifically, the unique region of DNA (i) will generally comprise a bacterial, viral, yeast synthetic, or other DNA which is not normally present in the
25 mammalian cell genome and which further does not comprise significant homology or sequence identity to DNA contained in the genome of the mammalian cell.

Essentially, this sequence should be sufficiently different to mammalian DNA that it will not significantly recombine with the host cell genome via homologous recombination. The size of such unique DNA 5 will generally be at least about 2 to 10 kilobases in size, or higher, more preferably at least about 10kb, as several other investigators have noted an increased frequency of targeted recombination as the size of the homology region is increased (Capecchi, *Science*, 10 244:1288-1292 (1989)).

The upper size limit of the unique DNA which acts as a site for homologous recombination with a sequence in the second target vector is largely dictated by potential stability constraints (if DNA is too large it 15 may not be easily integrated into a chromosome and the difficulties in working with very large DNAs.

(ii) a DNA including a fragment of a selectable marker DNA, typically an exon of a dominant selectable marker gene. The only essential feature of this DNA is 20 that it not encode a functional selectable marker protein unless it is expressed in association with a sequence contained in the target plasmid. Typically, the target plasmid will comprise the remaining exons of the dominant selectable marker gene (those not comprised in 25 "targeting" plasmid). Essentially, a functional selectable marker should only be produced if homologous recombination occurs (resulting in the association and

expression of this marker DNA (i) sequence together with the portion(s) of the selectable marker DNA fragment which is (are) contained in the target plasmid).

As noted, the current invention exemplifies the
5 use of the neomycin phosphotransferase gene as the dominant selectable marker which is "split" in the two vectors. However, other selectable markers should also be suitable, e.g., the *Salmonella histidinol dehydrogenase* gene, *hygromycin phosphotransferase* gene, *herpes simplex* 10 virus thymidine kinase gene, adenosine deaminase gene, glutamine synthetase gene and hypoxanthine-guanine phosphoribosyl transferase gene.

(iii) a DNA which encodes a functional selectable marker protein, which selectable marker is different
15 from the selectable marker DNA (ii). This selectable marker provides for the successful selection of mammalian cells wherein the marker plasmid is successfully integrated into the cellular DNA. More preferably, it is desirable that the marker plasmid comprise two such
20 dominant selectable marker DNAs, situated at opposite ends of the vector. This is advantageous as it enables integrants to be selected using different selection agents and further enables cells which contain the entire vector to be selected. Additionally, one marker
25 can be an amplifiable marker to facilitate gene amplification as discussed previously. Any of the

dominant selectable marker listed in (ii) can be used as well as others generally known in the art.

Moreover, the marker plasmid may optionally further comprise a rare endonuclease restriction site. This is potentially desirable as this may facilitate cleavage.

If present, such rare restriction site should be situated close to the middle of the unique region that acts as a substrate for homologous recombination. Preferably such sequence will be at least about 12 nucleotides.

10 The introduction of a double stranded break by similar methodology has been reported to enhance the frequency of homologous recombination. (Choulika et al, *Mol. Cell. Biol.*, 15:1968-1973 (1995)). However, the presence of such sequence is not essential.

15 The "targeting plasmid" will comprise at least the following sequences:

(1) the same unique region of DNA contained in the marker plasmid or one having sufficient homology or sequence identity therewith that said DNA is capable of combining via homologous recombination with the unique region (i) in the marker plasmid. Suitable types of DNAs are described *supra* in the description of the unique region of DNA (1) in the marker plasmid.

20 (2) The remaining exons of the dominant selectable marker, one exon of which is included as (ii) in the marker plasmid listed above. The essential features of this DNA fragment is that it result in a functional

(selectable) marker protein only if the target plasmid integrates via homologous recombination (wherein such recombination results in the association of this DNA with the other fragment of the selectable marker DNA contained in the marker plasmid) and further that it allow for insertion of a desired exogenous DNA. Typically, this DNA will comprise the remaining exons of the selectable marker DNA which are separated by an intron. For example, this DNA may comprise the first two exons of the neo gene and the marker plasmid may comprise the third exon (back third of neo).

(3) The target plasmid will also comprise a desired DNA, e.g., one encoding a desired polypeptide, preferably inserted within the selectable marker DNA fragment contained in the plasmid. Typically, the DNA will be inserted in an intron which is comprised between the exons of the selectable marker DNA. This ensures that the desired DNA is also integrated if homologous recombination of the target plasmid and the marker plasmid occurs. This intron may be naturally occurring or it may be engineered into the dominant selectable marker DNA fragment.

This DNA will encode any desired protein, preferably one having pharmaceutical or other desirable properties. Most typically the DNA will encode a mammalian protein, and in the current examples provided, an immunoglobulin or an immunoadhesin. However the

invention is not in any way limited to the production of immunoglobulins.

As discussed previously, the subject cloning method is suitable for any mammalian cell as it does not require for efficacy that any specific mammalian sequence or sequences be present. In general, such mammalian cells will comprise those typically used for protein expression, e.g., CHO cells, myeloma cells, COS cells, BHK cells, Sp2/0 cells, NIH 3T3 and HeLa cells. In the examples which follow, CHO cells were utilized. The advantages thereof include the availability of suitable growth medium, their ability to grow efficiently and to high density in culture, and their ability to express mammalian proteins such as immunoglobulins in biologically active form.

Further, CHO cells were selected in large part because of previous usage of such cells by the inventors for the expression of immunoglobulins (using the translationally impaired dominant selectable marker containing vectors described previously). Thus, the present laboratory has considerable experience in using such cells for expression. However, based on the examples which follow, it is reasonable to expect similar results will be obtained with other mammalian cells.

In general, transformation or transfection of mammalian cells according to the subject invention will be effected according to conventional methods. So that the

invention may be better understood, the construction of exemplary vectors and their usage in producing integrants is described in the examples below.

EXAMPLE 1

5

Design and Preparation of Marker
and Targeting Plasmid DNA Vectors

The marker plasmid herein referred to as "Desmond" was assembled from the following DNA elements:

(a) Murine dihydrofolate reductase gene (DHFR),
10 incorporated into a transcription cassette, comprising the mouse beta globin promoter 5" to the DHFR start site, and bovine growth hormone poly adenylation signal 3" to the stop codon. The DHFR transcriptional cassette was isolated from TCAE6, an expression vector created 15 previously in this laboratory (Newman et al, 1992, *Bio-technology*, 10:1455-1460).

(b) E. coli β-galactosidase gene - commercially available, obtained from Promega as pSV-b-galactosidase control vector, catalog # E1081.

20 (c) Baculovirus DNA, commercially available, purchased from Clontech as pBAKPAK8, cat # 6145-1.

(d) Cassette comprising promoter and enhancer elements from Cytomegalovirus and SV40 virus. The cassette was generated by PCR using a derivative of expression 25 vector TCAE8 (Reff et al, *Blood*, 83:435-445 (1994)). The enhancer cassette was inserted within the baculo-

virus sequence, which was first modified by the insertion of a multiple cloning site.

(e) E. coli GUS (glucuronidase) gene, commercially available, purchased from Clontech as pB101, cat. # 5 6017-1.

(f) Firefly luciferase gene, commercially available, obtained from Promega as pGEM-Luc (catalog # E1541).

(g) S. typhimurium histidinol dehydrogenase gene 10 (HisD). This gene was originally a gift from (Donahue et al, Gene, 18:47-59 (1982)), and has subsequently been incorporated into a transcription cassette comprising the mouse beta globin major promoter 5' to the gene, and the SV40 polyadenylation signal 3' to the gene.

15 The DNA elements described in (a)-(g) were combined into a pBR derived plasmid backbone to produce a 7.7kb contiguous stretch of DNA referred to in the attached figures as "homology". Homology in this sense refers to sequences of DNA which are not part of the mammalian genome and are used to promote homologous recombination 20 between transfected plasmids sharing the same homology DNA sequences.

(h) Neomycin phosphotransferase gene from TNS (Davis and Smith, Ann. Rev. Micro., 32:469-518 (1978)).

25 The complete neo gene was subcloned into pBluescript SK- (Stratagene catalog # 212205) to facilitate genetic manipulation. A synthetic linker was then inserted into

a unique PstI site occurring across the codons for amino acid 51 and 52 of neo. This linker encoded the necessary DNA elements to create an artificial splice donor site, intervening intron and splice acceptor site within the neo gene, thus creating two separate exons, presently referred to as neo exon 1 and 2. Neo exon 1 encodes the first 51 amino acids of neo, while exon 2 encodes the remaining 203 amino acids plus the stop codon of the protein A NotI cloning site was also created within the 10 intron.

Neo exon 2 was further subdivided to produce neo exons 2 and 3. This was achieved as follows: A set of PCR primers were designed to amplify a region of DNA encoding neo exon 1, intron and the first 111 2/3 amino acids of exon2. The 3' PCR primer resulted in the introduction of a new 5' splice site immediately after the second nucleotide of the codon for amino acid 111 in exon 2, therefore generating a new smaller exon 2. The DNA fragment now encoding the original exon 1, intron 15 and new exon 2 was then subcloned and propagated in a pBR based vector. The remainder of the original exon 2 was used as a template for another round of PCR amplification, which generated "exon3". The 5' primer for this round of amplification introduced a new splice acceptor site at the 5' side of the newly created exon 25 3, i.e. before the final nucleotide of the codon for amino acid 111. The resultant 3 exons of neo encode the

following information: exon 1 - the first 51 amino acids of neo; exon 2 - the next 111 2/3 amino acids, and exon 3 the final 91 1/3 amino acids plus the translational stop codon of the neo gene.

5 Neo exon 3 was incorporated along with the above mentioned DNA elements into the marking plasmid "Desmond". Neo exons 1 and 2 were incorporated into the targeting plasmid "Molly". The Not1 cloning site created within the intron between exons 1 and 2 was used in
10 subsequent cloning steps to insert genes of interest into the targeting plasmid.

A second targeting plasmid "Mandy" was also generated. This plasmid is almost identical to "Molly" (some restriction sites on the vector have been changed) except that the original HisD and DHFR genes contained in "Molly" were inactivated. These changes were incorporated because the Desmond cell line was no longer being cultured in the presence of Histidinol, therefore it seemed unnecessary to include a second copy of the
15 HisD gene. Additionally, the DHFR gene was inactivated to ensure that only a single DHFR gene, namely the one present in the Desmond marked site, would be amplifiable in any resulting cell lines. "Mandy" was derived from "Molly" by the following modifications:
20

25 (i) A synthetic linker was inserted in the middle of the DHFR coding region. This linker created a stop codon and shifted the remainder of the DHFR coding

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region out of frame, therefore rendering the gene nonfunctional.

(ii) A portion of the HisD gene was deleted and replaced with a PCR generated HisD fragment lacking the promoter and start codon of the gene.

Figure 1 depicts the arrangement of these DNA elements in the marker plasmid "Desmond". Figure 2 depicts the arrangement of these elements in the first targeting plasmid, "Molly". Figure 3 illustrates the possible arrangement in the CHO genome, of the various DNA elements after targeting and integration of Molly DNA into Desmond marked CHO cells. Figure 9 depicts the targeting plasmid "Mandy."

Construction of the marking and targeting plasmids from the above listed DNA elements was carried out following conventional cloning techniques (see, e.g., Molecular Cloning, A Laboratory Manual, J. Sambrook et al, 1987, Cold Spring Harbor Laboratory Press, and Current Protocols in Molecular Biology, F. M. Ausubel et al, eds., 1987, John Wiley and Sons). All plasmids were propagated and maintained in E. coli XLI blue (Stratagene, cat. # 200236). Large scale plasmid preparations were prepared using Promega Wizard Maxiprep DNA Purification System®, according to the manufacturer's directions.

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EXAMPLE 2

Construction of a Marked CHO Cell Line

1. Cell Culture and Transfection Procedures to
Produced Marked CHO Cell Line

5 Marker plasmid DNA was linearized by digestion overnight at 37°C with Bst1107I. Linearized vector was ethanol precipitated and resuspended in sterile TE to a concentration of 1mg/ml. Linearized vector was introduced into DHFR-Chinese hamster ovary cells (CHO cells)
10 DG44 cells (Urlaub et al, *Som. Cell and Mol. Gen.*,
12:555-566 (1986)) by electroporation as follows...

Exponentially growing cells were harvested by centrifugation, washed once in ice cold SBS (sucrose buffered solution, 272mM sucrose, 7mM sodium phosphate, pH 7.4, 1mM magnesium chloride) then resuspended in SBS to a concentration of 10⁷ cells/ml. After a 15 minute incubation on ice, 0.4ml of the cell suspension was mixed with 40µg linearized DNA in a disposable electroporation cuvette. Cells were shocked using a BTX electrocell manipulator (San Diego, CA) set at 230 volts, 400 microfaraday capacitance, 13 ohm resistance. Shocked cells were then mixed with 20 ml of prewarmed CHO growth media (CHO-S-SFMII, Gibco/BRL, catalog # 31033-012) and plated in 96 well tissue culture plates.
20 Forty eight hours after electroporation, plates were fed with selection media (in the case of transfection with Desmond, selection media is CHO-S-SFMII without

hypoxanthine or thymidine, supplemented with 2mM Histidinol (Sigma catalog # H6647)). Plates were maintained in selection media for up to 30 days, or until some of the wells exhibited cell growth. These cells
5 were then removed from the 96 well plates and expanded ultimately to 120 ml spinner flasks where they were maintained in selection media at all times.

EXAMPLE 3

Characterization of Marked CHO Cell Lines

10 (a) Southern Analysis

Genomic DNA was isolated from all stably growing Desmond marked CHO cells. DNA was isolated using the Invitrogen Easy® DNA kit, according to the manufacturer's directions. Genomic DNA was then digested with HindIII overnight at 37°C, and subjected to Southern analysis using a PCR generated digoxigenin labelled probe specific to the DHFR gene. Hybridizations and washes were carried out using Boehringer Mannheim's DIG easy hyb (catalog # 1603 558) and DIG Wash and Block Buffer Set (catalog # 1585 762) according to the manufacturer's directions. DNA samples containing a single band hybridizing to the DHFR probe were assumed to be Desmond clones arising from a single cell which had integrated a single copy of the plasmid. These clones
15 were retained for further analysis. Out of a total of 45 HisD resistant cell lines isolated, only 5 were
20
25

single copy integrants. Figure 4 shows a Southern blot containing all 5 of these single copy Desmond clones. Clone names are provided in the figure legend.

(b) Northern Analysis

5 Total RNA was isolated from all single copy Desmond clones using TRIzol reagent (Gibco/BRL cat # 15596-026) according to the manufacturer's directions. 10-20 μ g RNA from each clone was analyzed on duplicate formaldehyde gels. The resulting blots were probed with PCR generated digoxigenin labelled DNA probes to (i) DHFR message, (ii) HisD message and (iii) CAD message. CAD is a trifunctional protein involved in uridine biosynthesis (Wahl et al, *J. Biol. Chem.*, 254, 17:8679-8689 (1979)), and is expressed equally in all cell types. It is used here as an internal control to help quantitate RNA loading. Hybridizations and washes were carried out using the above mentioned Boehringer Mannheim reagents. The results of the Northern analysis are shown in Figure 5. The single copy Desmond clone exhibiting the highest levels of both the His D and DHFR message is clone 15C9, shown in lane 4 in both panels of the figure. This clone was designated as the "marked cell line" and used in future targeting experiments in CHO, examples of which are presented in the following sections.

EXAMPLE 4Expression of Anti-CD20 Antibody
in Desmond Marked CHO Cells

C2B8, a chimeric antibody which recognizes B-cell surface antigen CD20, has been cloned and expressed previously in our laboratory. (Reff et al, *Blood*, 83:434-45 (1994)). A 4.1 kb DNA fragment comprising the C2B8 light and heavy chain genes, along with the necessary regulatory elements (eukaryotic promoter and polyadenylation signals) was inserted into the artificial intron created between exons 1 and 2 of the neo gene contained in a pBR derived cloning vector. This newly generated 5kb DNA fragment (comprising neo exon 1, C2B8 and neo exon 2) was excised and used to assemble the targeting plasmid Molly. The other DNA elements used in the construction of Molly are identical to those used to construct the marking plasmid Desmond, identified previously. A complete map of Molly is shown in Fig. 2.

The targeting vector Molly was linearized prior to transfection by digestion with *Kpn*1 and *Pac*1, ethanol precipitated and resuspended in sterile TE to a concentration of 1.5mg/mL. Linearized plasmid was introduced into exponentially growing Desmond marked cells essentially as described, except that 80 μ g DNA was used in each electroporation. Forty eight hours postelectroporation, 96 well plates were supplemented with selection medium - CHO-SSFMII supplemented with 400 μ g/mL Geneti-

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cin (G418, Gibco/BRL catalog # 10131-019). Plates were maintained in selection medium for up to 30 days, or until cell growth occurred in some of the wells. Such growth was assumed to be the result of clonal expansion of a single G418 resistant cell. The supernatants from all G418 resistant wells were assayed for C2B8 production by standard ELISA techniques, and all productive clones were eventually expanded to 120mL spinner flasks and further analyzed.

10 Characterization of Antibody secreting Targeted Cells

A total of 50 electroporations with Molly targeting plasmid were carried out in this experiment, each of which was plated into separate 96 well plates. A total of 10 viable, anti-CD20 antibody secreting clones were obtained and expanded to 120ml spinner flasks. Genomic DNA was isolated from all clones, and Southern analyses were subsequently performed to determine whether the clones represented single homologous recombination events or whether additional random integrations had occurred in the same cells. The methods for DNA isolation and Southern hybridization were as described in the previous section. Genomic DNA was digested with EcoRI and probed with a PCR generated digoxigenin labelled probe to a segment of the CD20 heavy chain constant region. The results of this Southern analysis are presented in figure 6. As can be seen in the figure, 8 of

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the 10 clones show a single band hybridizing to the CD20 probe, indicating a single homologous recombination event has occurred in these cells. Two of the ten, clones 24G2 and 28C9, show the presence of additional 5 band(s), indicative of an additional random integration elsewhere in the genome.

We examined the expression levels of anti-CD20 antibody in all ten of these clones, the data for which is shown in Table 1, below.

10

Table 1:

Expression Level of Anti-CD20
Secreting Homologous Integrants

	<u>Clone</u>	<u>Anti-CD20, pg/c/d</u>
15	20F4	3.5
	25E1	2.4
	42F9	1.8
	39G11	1.5
	21C7	1.3
	50G10	0.9
20	29F9	0.8
	5F9	0.3
<hr/>		
	28C9*	4.5
	24G2*	2.1

5

* These clones contained additional randomly integrated copies of anti-CD20. Expression levels of these clones therefore reflect a contribution from both the homologous and random sites.

Expression levels are reported as picogram per cell per day (pg/c/d) secreted by the individual clones, and represented the mean levels obtained from three separate ELISAs on samples taken from 120 mL spinner flasks.

10

As can be seen from the data, there is a variation in antibody secretion of approximately ten fold between the highest and lowest clones. This was somewhat unexpected as we anticipated similar expression levels from all clones due to the fact the anti-CD20 genes are all integrated into the same Desmond marked site. Nevertheless, this observed range in expression extremely small in comparison to that seen using any traditional random integration method or with our translationally impaired vector system.

15

Clone 20F4, the highest producing single copy integrant was selected for further study. Table 2 (below) presents ELISA and cell culture data from seven day production runs of this clone.

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Table 2:

7 Day Production Run Data for 20F4

Day	% Viable	Viable/ml (x 10 ⁵)	T _{x2} (hr)	mg/L	pg/c/d
1	96	3.4	31	1.3	4.9
5	2	94	6	2.5	3.4
	3	94	9.9	33	4.7
	4	90	17.4	30	6.8
	5	73	14		8.3
	6	17	3.5		9.5

10 Clone 20F4 was seeded at 2x10⁵ml in a 120ml spinner flask on day 0. On the following six days, cell counts were taken, doubling times calculated and 1ml samples of supernatant removed from the flask and analyzed for secreted anti-CD20 by ELISA.

15 This clone is secreting on average, 3-5pg antibody/- cell/day, based on this ELISA data. This is the same level as obtained from other high expressing single copy clones obtained previously in our laboratory using the previously developed translationally impaired random 20 integration vectors. This result indicates the following:

25 (1) that the site in the CHO genome marked by the Desmond marking vector is highly transcriptionally active, and therefore represents an excellent site from which to express recombinant proteins, and

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(2) that targeting by means of homologous recombination can be accomplished using the subject vectors and occurs at a frequency high enough to make this system a viable and desirable alternative to random integration methods.

To further demonstrate the efficacy of this system, we have also demonstrated that this site is amplifiable, resulting in even higher levels of gene expression and protein secretion. Amplification was achieved by plating serial dilutions of 20F4 cells, starting at a density of 2.5×10^4 cells/ml, in 96 well tissue culture dishes, and culturing these cells in media (CHO-SSFMII) supplemented with 5, 10, 15 or 20nM methotrexate. Antibody secreting clones were screened using standard ELISA techniques, and the highest producing clones were expanded and further analyzed. A summary of this amplification experiment is presented in Table 3 below.

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Table 3:

Summary of 20F4 Amplification

nM MTX	# Wells Assayed	Expression Level mg/l 96 well	# Wells Expanded	Expression Level pg/c/d from spinner
10	56	3-13	4	10-15
15	27	2-14	3	15-18
20	17	4-11	1	ND

Methotrexate amplification of 20F4 was set up as described in the text, using the concentrations of methotrexate indicated in the above table. Supernatants from all surviving 96 well colonies were assayed by ELISA, and the range of anti-CD20 expressed by these clones is indicated in column 3. Based on these results, the highest producing clones were expanded to 120ml spinners and several ELISAs conducted on the spinner supernatants to determine the pg/cell/day expression levels, reported in column 5.

The data here clearly demonstrates that this site can be amplified in the presence of methotrexate. Clones from the 10 and 15nM amplifications were found to produce on the order of 15-20pg/cell/day.

A 15nM clone, designated 20F4-15A5, was selected as the highest expressing cell line. This clone originated from a 96 well plate in which only 22 wells grew, and was therefore assumed to have arisen from a single cell. A 15nM clone, designated 20F4-15A5, was selected as the highest expressing cell line. This clone originated

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from a 96 well plate in which only 22 wells grew, and was therefore assumed to have arisen from a single cell. The clone was then subjected to a further round of methotrexate amplification. As described above, serial 5 dilutions of the culture were plated into 96 well dishes and cultured in CHO-SS-FMII medium supplemented with 200, 300 or 400nM methotrexate. Surviving clones were screened by ELISA, and several high producing clones were expanded to spinner cultures and further analyzed. 10 A summary of this second amplification experiment is presented in Table 4.

Table 4:
Summary of 20F4-15A5 Amplification

nM MTX	# Wells Assayed	Expression Level mg/l 96 well	# Wells Expanded	Expression Level pg/c/d, spinner
15 200	67	23-70	1	50-60
250	86	21-70	4	55-60
300	81	15-75	3	40-50

20 Methotrexate amplifications of 20F4-15A5 were set up and assayed as described in the text. The highest producing wells, the numbers of which are indicated in column 4, were expanded to 120ml spinner flasks. The expression levels of the cell lines derived from these wells is recorded as pg/c/d in column 5.

25 The highest producing clone came from the 250nM methotrexate amplification. The 250nM clone, 20F4-15A5-250A6 originated from a 96 well plate in which only wells

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grew, and therefore is assumed to have arisen from a single cell. Taken together, the data in Tables 3 and 4 strongly indicates that two rounds of methotrexate amplification are sufficient to reach expression levels of 5 60pg/cell/day, which is approaching the maximum secretion capacity of immunoglobulin in mammalian cells (Reff, M.E., *Curr. Opin. Biotech.*, 4:573-576 (1993)). The ability to reach this secretion capacity with just two amplification steps further enhances the utility of 10 this homologous recombination system. Typically, random integration methods require more than two amplification steps to reach this expression level and are generally less reliable in terms of the ease of amplification. Thus, the homologous system offers a more efficient and 15 time saving method of achieving high level gene expression in mammalian cells.

EXAMPLE 5

Expression of Anti-Human CD23 Antibody
in Desmond Marked CHO Cells

20 CD23 is low affinity IgE receptor which mediates binding of IgE to B and T lymphocytes (Sutton, B.J., and Gould, H.J., *Nature*, 366:421-428 (1993)). Anti-human CD23 monoclonal antibody 5E8 is a human gamma-1 monoclonal antibody recently cloned and expressed in our 25 laboratory. This antibody is disclosed in commonly

assigned Serial No. 08/803,085, filed on February 20, 1997.

The heavy and light chain genes of 5E8 were cloned into the mammalian expression vector N5KG1, a derivative 5 of the vector NEOSPLA (Barnett et al., in *Antibody Expression and Engineering*, H.Y Yang and T. Imanaka, eds., pp27-40 (1995)) and two modifications were then made to the genes. We have recently observed somewhat higher secretion of immunoglobulin light chains compared to 10 heavy chains in other expression constructs in the laboratory (Reff et al, 1997, unpublished observations). In an attempt to compensate for this deficit, we altered 15 the 5E8 heavy chain gene by the addition of a stronger promoter/enhancer element immediately upstream of the start site. In subsequent steps, a 2.9kb DNA fragment comprising the 5E8 modified light and heavy chain genes was isolated from the N5KG1 vector and inserted into the targeting vector Mandy. Preparation of 5E8-containing Molly and electroporation into Desmond 15C9 CHO cells 20 was essentially as described in the preceding section.

One modification to the previously described protocol was in the type of culture medium used. Desmond marked CHO cells were cultured in protein-free CD-CHO medium (Gibco-BRL, catalog # AS21206) supplemented with 25 3mg/L recombinant insulin (3mg/mL stock, Gibco-BRL, catalog # AS22057) and 8mM L-glutamine (200mM stock, Gibco-BRL, catalog # 25030-081). Subsequently, trans-

fected cells were selected in the above medium supplemented with 400 μ g/mL geneticin. In this experiment, 20 electroporations were performed and plated into 96 well tissue culture dishes. Cells grew and secreted anti-

5 CD23 in a total of 68 wells, all of which were assumed to be clones originating from a single G418 cell.

Twelve of these wells were expanded to 120ml spinner flasks for further analysis. We believe the increased number of clones isolated in this experiment (68 com-

10 pared with 10 for anti-CD20 as described in Example 4) is due to a higher cloning efficiency and survival rate of cells grown in CD-CHO medium compared with CHO-SS-FMII medium. Expression levels for those clones analyzed in spinner culture ranged from 0.5-3pg/c/d, in

15 close agreement with the levels seen for the anti-CD20 clones. The highest producing anti-CD23 clone, designated 4H12, was subjected to methotrexate amplification in order to increase its expression levels. This amplification was set up in a manner similar to that described

20 for the anti-CD20 clone in Example 4. Serial dilutions of exponentially growing 4H12 cells were plated into 96 well tissue culture dishes and grown in CD-CHO medium supplemented with 3mg/L insulin, 8mM glutamine and 30, 35 or 40nM methotrexate. A summary of this

25 amplification experiment is presented in Table 5.

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Summary of 2H12 Amplification

nM MTX	# Wells Assayed	Expression Level mg/l 96 well	# Wells Expanded	Expression Level pg/c/d from spinner
30	100	6-24	8	10-25
35	64	4-27	2	10-15
5	40	4-20	1	ND

The highest expressing clone obtained was a 30nM clone, isolated from a plate on which 22 wells had grown. This clone, designated 4H12-30G5, was reproducibly secreting 18-22pg antibody per cell per day. This is the same range of expression seen for the first amplification of the anti CD20 clone 20F4 (clone 20F4-15A5 which produced 15-18pg/c/d, as described in Example 4). This data serves to further support the observation that amplification at this marked site in CHO is reproducible and efficient. A second amplification of this 30nM cell line is currently underway. It is anticipated that saturation levels of expression will be achievable for the anti-CD23 antibody in just two amplification steps, as was the case for anti-CD20.

20

EXAMPLE 6

Expression of Immunoadhesin in Desmond Marked CHO Cells

CTLA-4, a member of the Ig superfamily, is found on the surface of T lymphocytes and is thought to play a role in antigen-specific T-cell activation (Dariavach et al, *Eur. J. Immunol.*, 18:1901-1905 (1988); and Linsley et al, *J. Exp. Med.*, 174:561-569 (1991)). In order to further study the precise role of the CTLA-4 molecule in the activation pathway, a soluble fusion protein comprising the extracellular domain of CTLA-4 linked to a truncated form of the human IgG1 constant region was

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created (Linsley et al (Id.). We have recently expressed this CTLA-4 Ig fusion protein in the mammalian expression vector BLECH1, a derivative of the plasmid NEOSPLA (Barnett et al, in Antibody Expression and Engineering, H.Y Yang and T. Imanaka, eds., pp27-40 (1995)). An 800bp fragment encoding the CTLA-4 Ig was isolated from this vector and inserted between the SacII and BglII sites in Molly.

Preparation of CTLA-4Ig-Molly and electroporation into Desmond clone 15C9 CHO cells was performed as described in the previous example relating to anti-CD20. Twenty electroporations were carried out, and plated into 96 well culture dishes as described previously. Eighteen CTLA-4 expressing wells were isolated from the 96 well plates and carried forward to the 120ml spinner stage. Southern analyses on genomic DNA isolated from each of these clones were then carried out to determine how many of the homologous clones contained additional random integrants. Genomic DNA was digested with BglII and probed with a PCR generated digoxigenin labelled probe to the human IgG1 constant region. The results of this analysis indicated that 85% of the CTLA-4 clones are homologous integrants only; the remaining 15% contained one additional random integrant. This result corroborates the findings from the expression of anti-CD20 discussed above, where 80% of the clones were single homologous integrants. Therefore, we can conclude

that this expression system reproducibly yields single targeted homologous integrants in at least 80% of all clones produced.

Expression levels for the homologous CTLA4-Ig clones ranged from 8-12 pg/cell/day. This is somewhat higher than the range reported for anti-CD20 antibody and anti-CD23 antibody clones discussed above. However, we have previously observed that expression of this molecule using the intronic insertion vector system also resulted in significantly higher expression levels than are obtained for immunoglobulins. We are currently unable to provide an explanation for this observation.

EXAMPLE 7

Targeting Anti-CD20 to an alternate Desmond Marked CHO Cell Line

As we described in a preceding section, we obtained 5 single copy Desmond marked CHO cell lines (see Figures 4 and 5). In order to demonstrate that the success of our targeting strategy is not due to some unique property of Desmond clone 15C9 and limited only to this clone, we introduced anti-CD20 Molly into Desmond clone 9B2 (lane 6 in figure 4, lane 1 in figure 5). Preparation of Molly DNA and electroporation into Desmond 9B2 was exactly as described in the previous example pertaining to anti-CD20. We obtained one homologous integrant from this experiment. This clone was expanded to a 120ml

spinner flask, where it produced on average 1.2pg anti-CD20/cell/day. This is considerably lower expression than we observed with Molly targeted into Desmond 15C9. However, this was the anticipated result, based on our 5 northern analysis of the Desmond clones. As can be seen in Figure 5, mRNA levels from clone 9B2 are considerably lower than those from 15C9, indicating the site in this clone is not as transcriptionally active as that in 15C9. Therefore, this experiment not only demonstrates 10 the reproducibility of the system - presumably any marked Desmond site can be targeted with Molly - it also confirms the northern data that the site in Desmond 15C9 is the most transcriptionally active.

From the foregoing, it will be appreciated that, 15 although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without diverting from the scope of the invention. Accordingly, the invention is not limited by the appended claims.

WHAT IS CLAIMED IS:

1. A method for inserting a desired DNA at a target site in the genome of a mammalian cell which comprises the following steps:

5 (i) transfected or transforming a mammalian cell with a first plasmid ("marker plasmid") containing the following sequences:

10 (a) a region of DNA that is heterologous to the mammalian cell genome which when integrated in the mammalian cell genome provides a unique site for homologous recombination;

(b) a DNA fragment encoding a portion of a first selectable marker protein; and

15 (c) at least one other selectable marker DNA that provides for selection of mammalian cells which have been successfully integrated with the marker plasmid;

(ii) selecting a cell which contain the marker plasmid integrated in its genome;

20 (iii) transfected or transforming said selected cell with a second plasmid ("target plasmid") which contains the following sequences:

25 (a) a region of DNA that is identical or is sufficiently homologous to the unique region in the marker plasmid such that this region of DNA can recombine with said DNA via homologous recombination;

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(b) a DNA fragment encoding a portion of the same selectable marker contained in the marker plasmid, wherein the active selectable marker protein encoded by said DNA is only produced if said fragment is expressed
5 in association with the fragment of said selectable marker DNA contained in the marker plasmid; and

(iv) selecting cells which contain the target plasmid integrated at the target site by screening for the expression of the first selectable marker protein.

10 2. The method of Claim 1, wherein the DNA fragment encoding a fragment of a first selectable marker is an exon of a dominant selectable marker.

15 3. The method of Claim 2, wherein the second plasmid contains the remaining exons of said first selectable marker.

4. The method of Claim 3, wherein at least one DNA encoding a desired protein is inserted between said exons of said first selectable marker contained in the target plasmid.

20 5. The method of Claim 4, wherein a DNA encoding a dominant selectable marker is further inserted between the exons of said first selectable marker contained in

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the target plasmid to provide for co-amplification of the DNA encoding the desired protein.

6. The method of Claim 3, wherein the first dominant selectable marker is selected from the group consisting of neomycin phosphotransferase, histidinol dehydrogenase, dihydrofolate reductase, hygromycin phosphotransferase, herpes simplex virus thymidine kinase, adenosine deaminase, glutamine synthetase, and hypoxanthine-guanine phosphoribosyl transferase.

10 7. The method of Claim 4, wherein the desired protein is a mammalian protein.

8. The method of Claim 7, wherein the protein is an immunoglobulin.

15 9. The method of Claim 1, which further comprises determining the RNA levels of the selectable marker (c) contained in the marker plasmid prior to integration of the target vector.

20 10. The method of Claim 9, wherein the other selectable marker contained in the marker plasmid is a dominant selectable marker selected from the group consisting of histidinol dehydrogenase, herpes simplex

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thymidine kinase, hydromycin phosphotransferase, adenosine deaminase and glutamine synthetase.

11. The method of Claim 1, wherein the mammalian cell is selected from the group consisting of Chinese hamster ovary (CHO) cells, myeloma cells, baby hamster kidney cells, COS cells, NSO cells, HeLa cells and NIH 3T3 cells.

12. The method of Claim 11, wherein the cell is a CHO cell.

10 13. The method of Claim 1, wherein the marker plasmid contains the third exon of the neomycin phosphotransferase gene and the target plasmid contains the first two exons of the neomycin phosphotransferase gene.

15 14. The method of Claim 1, wherein the marker plasmid further contains a rare restriction endonuclease sequence which is inserted within the region of homology.

20 15. The method of Claim 1, wherein the unique region of DNA that provides for homologous recombination is a bacterial DNA, a viral DNA or a synthetic DNA.

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16. The method of Claim 1, wherein the unique region of DNA that provides for homologous recombination is at least 300 nucleotides.

17. The method of Claim 16, wherein the unique 5 region of DNA ranges in size from about 300 nucleotides to 20 kilobases.

18. The method of claim 17, wherein the unique region of DNA preferably ranges in size from 2 to 10 kilobases.

10 19. The method of Claim 1, wherein the first selectable marker DNA is split into at least three exons.

20. The method of Claim 1, wherein the unique region of DNA that provides for homologous recombination 15 is a bacterial DNA, an insect DNA, a viral DNA or a synthetic DNA.

21. The method of Claim 20, wherein the unique region of DNA does not contain any functional genes.

20 22. A vector system for inserting a desired DNA at a target site in the genome of a mammalian cell which comprises at least the following:

(i) a first plasmid ("marker plasmid") containing at least the following sequences:

(a) a region of DNA that is heterologous to the mammalian cell genome which when integrated in the mammalian cell genome provides a unique site for homologous recombination;

(b) a DNA fragment encoding a portion of a first selectable marker protein; and

(c) at least one other selectable marker DNA that provides for selection of mammalian cells which have been successfully integrated with the marker plasmid; and

(ii) a second plasmid ("target plasmid") which contains at least the following sequences:

(a) a region of DNA that is identical or is sufficiently homologous to the unique region in the marker plasmid such that this region of DNA can recombine with said DNA via homologous recombination;

(b) a DNA fragment encoding a portion of the same selectable marker contained in the marker plasmid, wherein the active selectable marker protein encoded by said DNA is only produced if said fragment is expressed in association with the fragment of said selectable marker DNA contained in the marker plasmid.

- 55 -

23. The vector system of Claim 22, wherein the DNA fragment encoding a fragment of a first selectable marker is an exon of a dominant selectable marker.

24. The vector system of Claim 23, wherein the
5 second plasmid contains the remaining exons of said
first selectable marker.

25. The vector system of Claim 24, wherein at
least one DNA encoding a desired protein is inserted
between said exons of said first selectable marker con-
10 tained in the target plasmid.

26. The vector system of Claim 24, wherein a DNA
encoding a dominant selectable marker is further insert-
ed between the exons of said first selectable marker
contained in the target plasmid to provide for co-ampli-
15 fication of the DNA encoding the desired protein.

27. The vector system of Claim 24, wherein the
first dominant selectable marker is selected from the
group consisting of neomycin phosphotransferase,
histidinol dehydrogenase, dihydrofolate reductase,
hygromycin phosphotransferase, herpes simplex virus
20 thymidine kinase, adenosine deaminase, glutamine synthe-
tase, and hypoxanthine-guanine phosphoribosyl transfer-
ase.

- 56 -

28. The vector system of Claim 25, wherein the desired protein is a mammalian protein.

29. The vector system of Claim 28, wherein the protein is an immunoglobulin.

5 30. The vector system of Claim 22, wherein the other selectable marker contained in the marker plasmid is a dominant selectable marker selected from the group consisting of histidinol dehydrogenase, herpes simplex thymidine kinase, hydromycin phosphotransferase, adenosine deaminase and glutamine synthetase.

10 31. The vector system of Claim 22, which provides for insertion of a desired DNA at a targeted site in the genome of a mammalian cell selected from the group consisting of Chinese hamster ovary (CHO) cells, myeloma cells, baby hamster kidney cells, COS cells, NSO cells, HeLa cells and NIH 3T3 cells.

15 32. The vector system of Claim 31, wherein the mammalian cell is a CHO cell.

20 33. The vector system of Claim 22, wherein the marker plasmid contains the third exon of the neomycin phosphotransferase gene and the target plasmid contains

- 57 -

the first two exons of the neomycin phosphotransferase gene.

34. The vector system of Claim 22, wherein the marker plasmid further contains a rare restriction endonuclease sequence which is inserted within the region of homology.

5 35. The vector system of Claim 22, wherein the unique region of DNA that provides for homologous recombination is a bacterial DNA, a viral DNA or a synthetic
10 DNA.

36. The vector system of Claim 22, wherein the unique region of DNA (a) contained in the marker plasmid vector system that provides for homologous recombination is at least 300 nucleotides.

15 37. The vector system of Claim 36, wherein the unique region of DNA ranges in size from about 300 nucleotides to 20 kilobases.

20 38. The vector system of Claim 37, wherein the unique region of DNA preferably ranges in size from 2 to 10 kilobases.

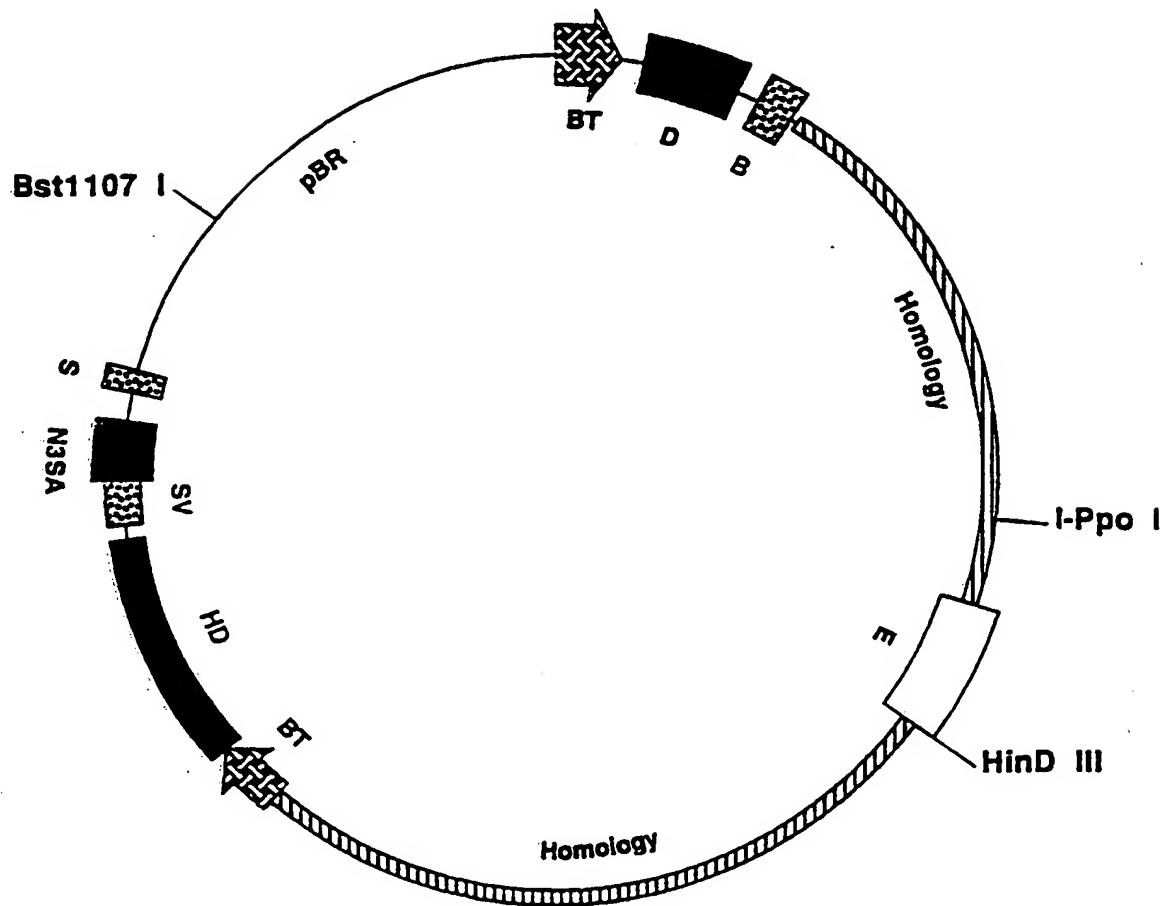
- 58 -

39. The vector system of Claim 22, wherein the first selectable marker DNA is split into at least three exons.

40. The vector system of Claim 22, wherein the
5 unique region of DNA that provides for homologous recombination is a bacterial DNA, an insect DNA, a viral DNA or a synthetic DNA.

41. The vector system of Claim 40, wherein the
unique region of DNA does not contain any functional
10 genes.

DESMOND



HD = *Salmonella HisD Gene*

N3 = *Neomycin Phosphotransferase Exon 3*

D = *Murine Dihydrofolate reductase*

E = *Cytomegalovirus and SV40 Enhancers*

SA = *Splice acceptor*

BT = *Mouse Beta Globin Major Promoter*

B = *Bovine Growth Hormone Polyadenylation*

S = *SV40 Early Polyadenylation*

SV = *SV40 Late Polyadenylation*

FIGURE 1A

Desmond

14,683 bp Bst1107 I linear

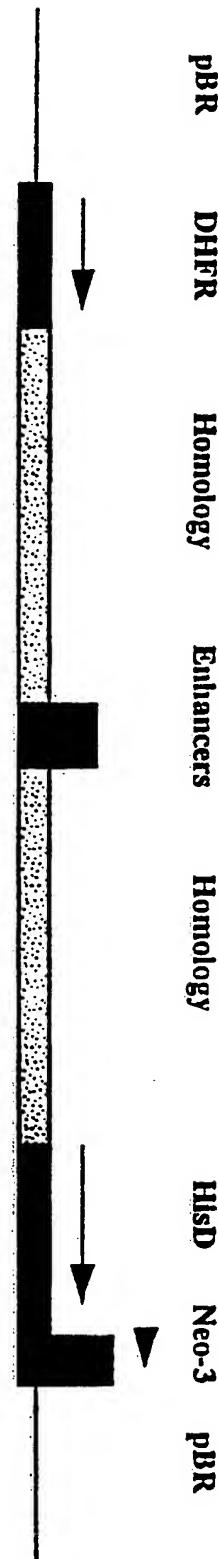
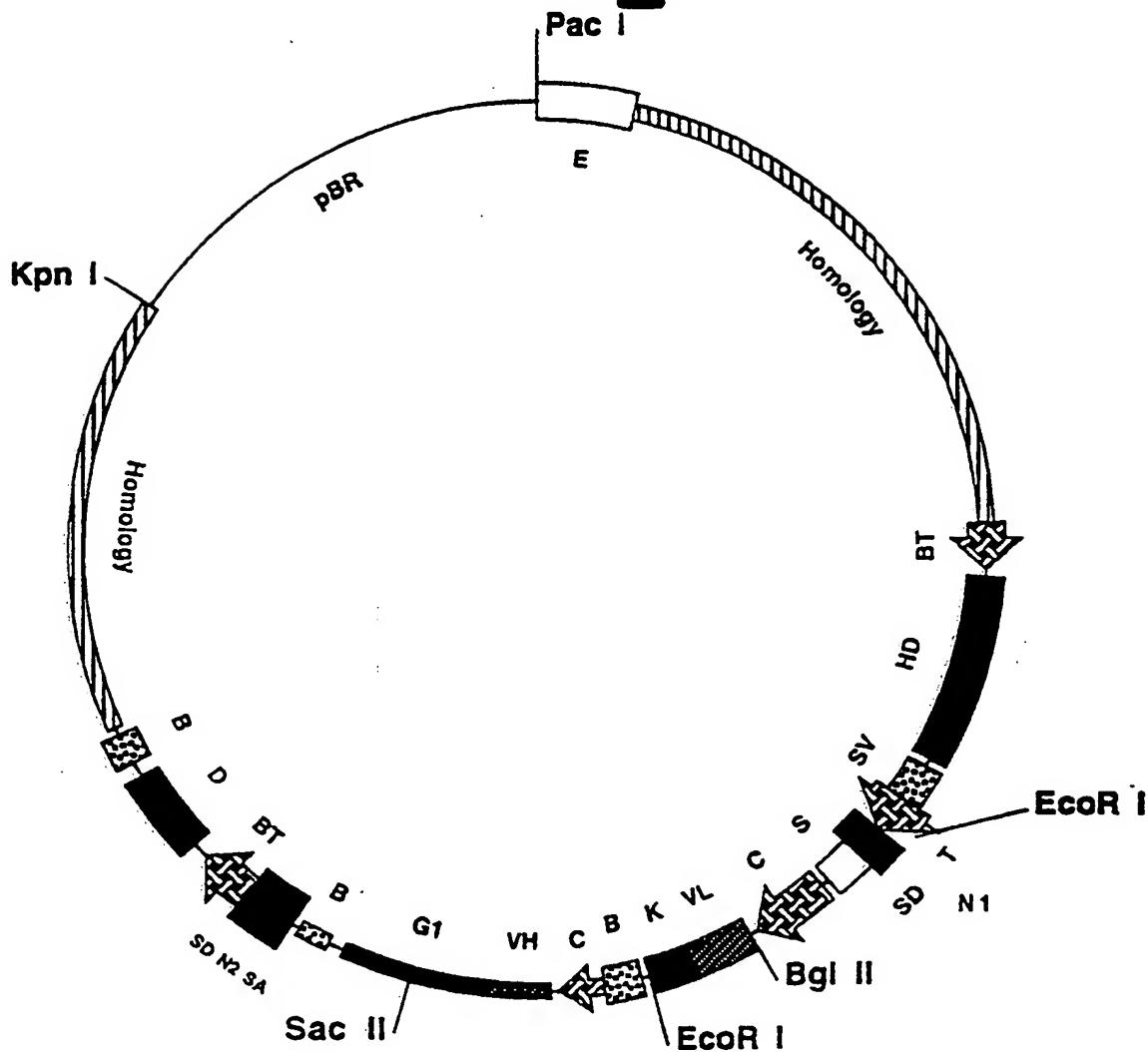


FIGURE 1B

Molly



D = Dihydrofolate reductase

N1 = Neomycin Phosphotransferase Exon 1

N2 = Neomycin Phosphotransferase Exon 2

VL = Anti-CD20 Light chain leader + Variable

K = Human Kappa Constant

VH = Anti-CD20 Heavy chain Leader + Variable

G1 = Human Gamma 1 Constant

HD = Salmonella Histidinol Dehydrogenase

E = CMV and SV40 enhancers S = SV40 Origin

SD = Splice donor SA = Splice acceptor

C = CMV promoter/enhancer

T = HSV TK promoter and Polyoma enhancers

BT = Mouse Beta Globin Major Promoter

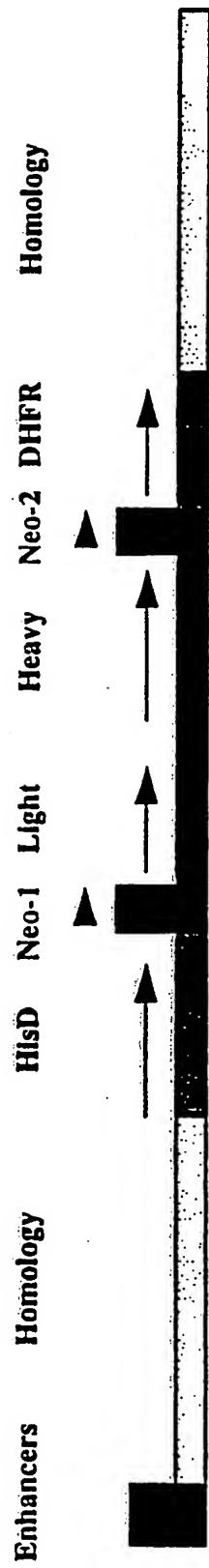
SV = SV40 Late Polyadenylation

B = Bovine Growth Hormone Polyadenylation

FIGURE 2A

Molly

15,987 bp Pac I, Kpn I fragment

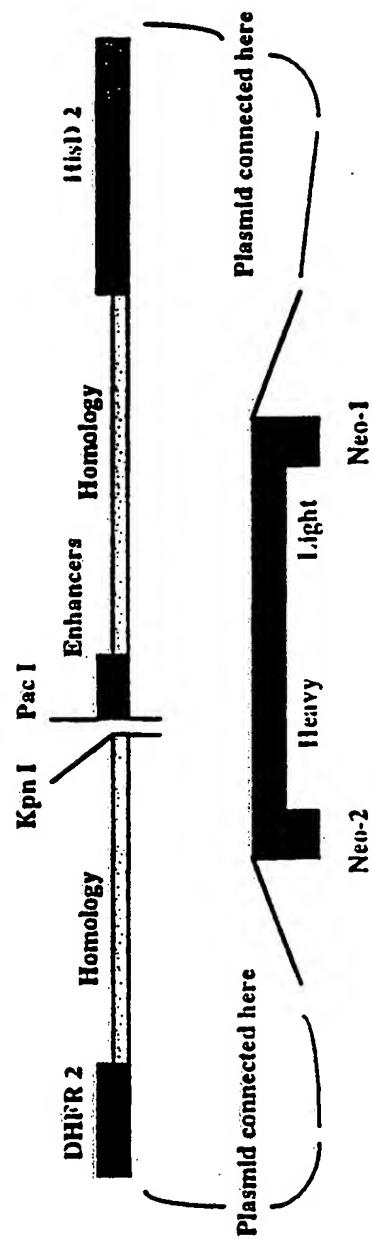


Homologous Recombination

Desmond in CHO



Molly



Single crossover in CHO



Southern Analysis of Desmond Marked CHO Cells

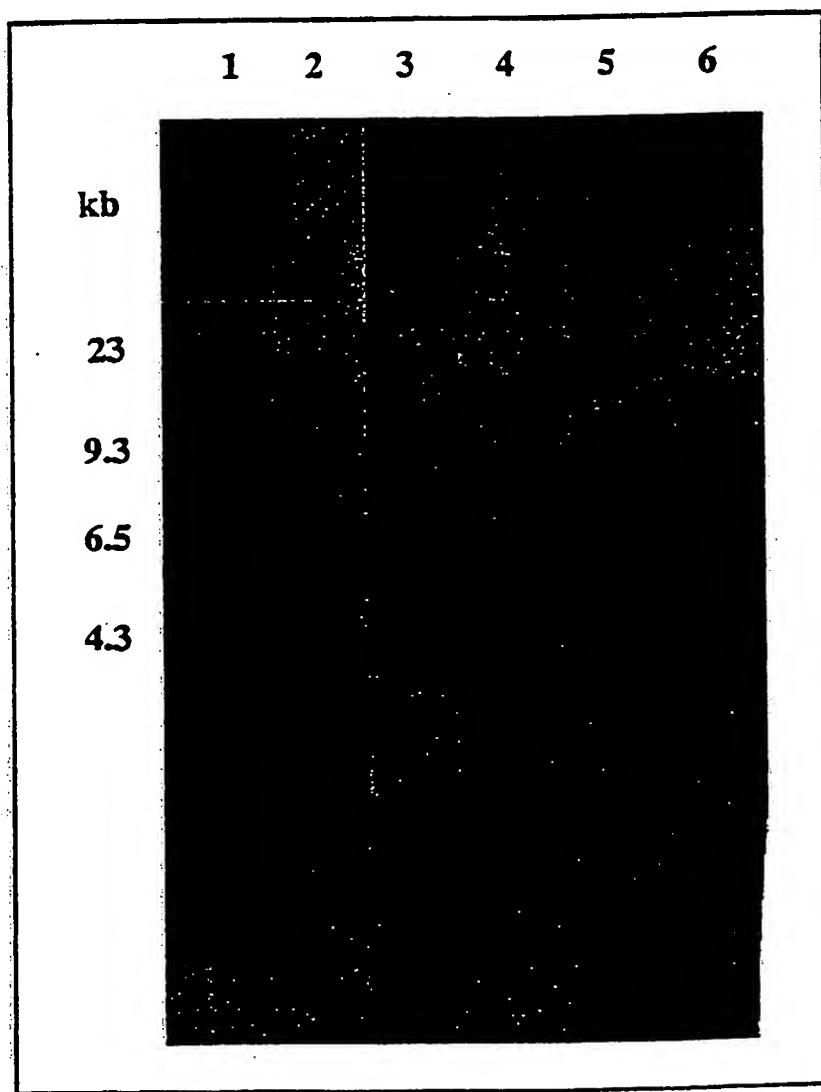


FIGURE 4

**Northern Analysis of Desmond
Marked CHO Cells**

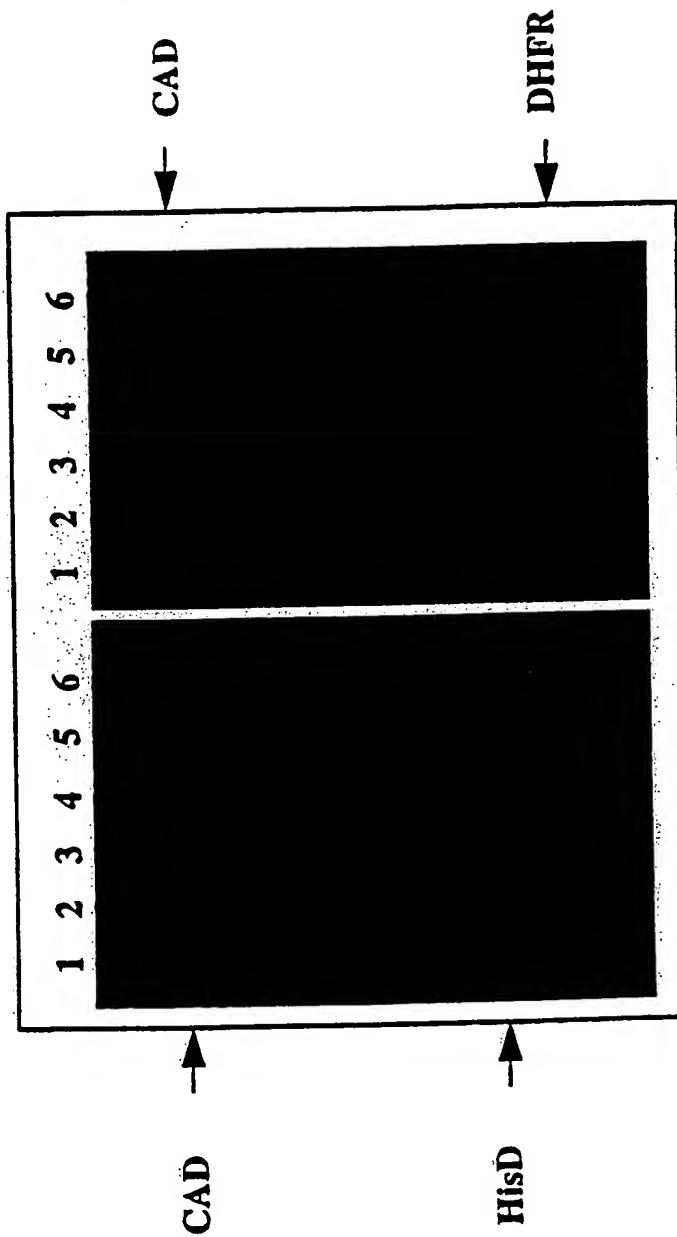


FIGURE 5

Southern Analysis of Anti CD20 Integrants in Marked CHO Cells

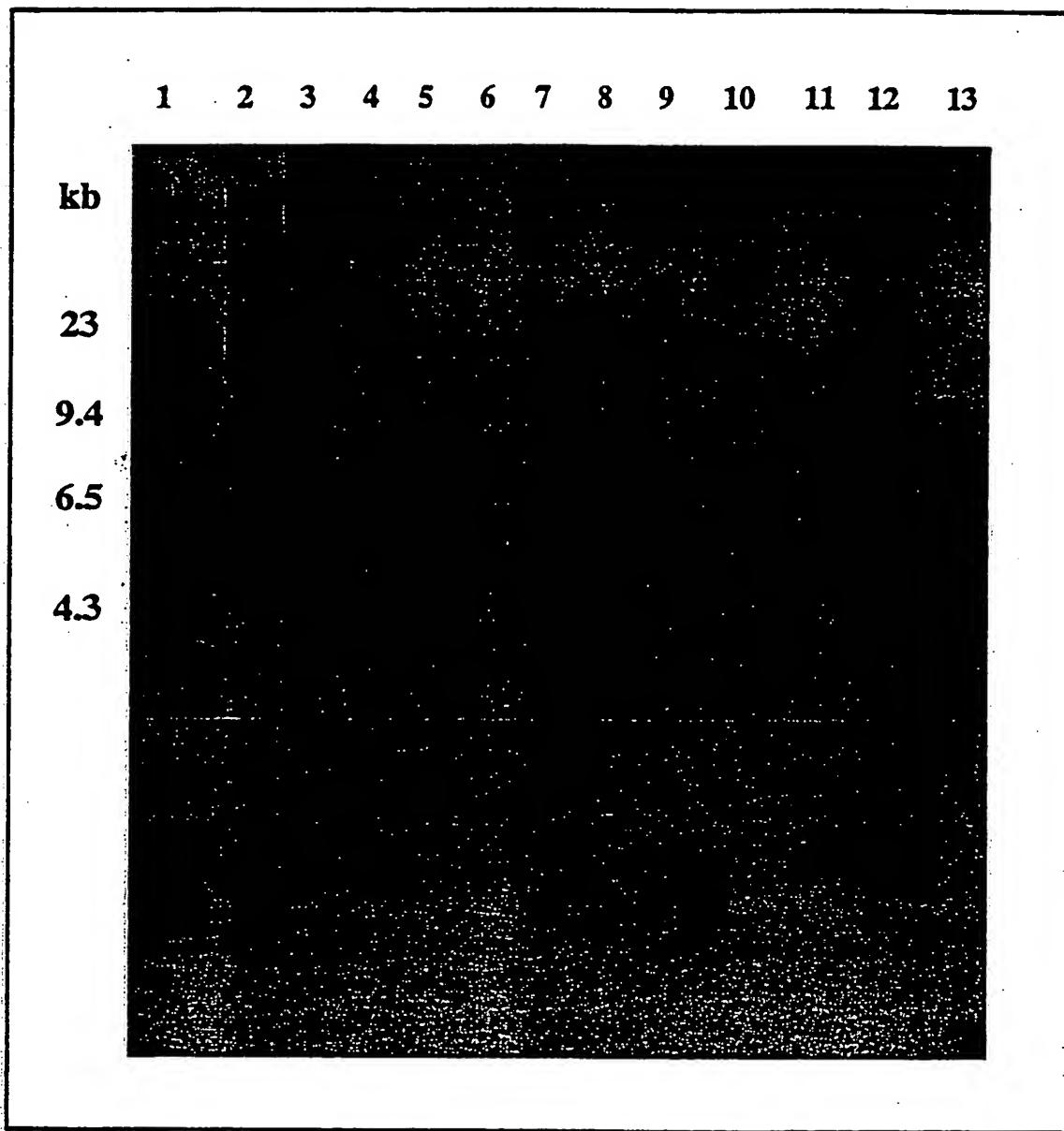


FIGURE 6

DNASIS
Desmond

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 70 80 90 100 110 120
 AGAAAAAAAG GAAAATTAAT TTTAACACCA ATTCAGTAGT TGATTGAGCA AATGCCTG
 130 140 150 160 170 180
 CAAAAAGGAT GCTTAGAGA CAGTGTTCTC TGACAGATA AGGACAAACA TTATTCAGAG
 190 200 210 220 230 240
 GGAGTACCCA GAGCTGAGAC TCCTAACCCA GTGAGTGGCA CAGCATCCAG GGAGAAATAT
 250 260 270 280 290 300
 GCTTGTATC ACCGAAGCCT GATTCCGTAG AGCCACACCC TGTTAAGGGC CAATCTGCTC
 310 320 330 340 350 360
 ACACAGGATA GAGAGGGCAG GAGCCAGGGC AGAGCATATA AGGTGAGGTA GGATCAGTTG
 370 380 390 400 410 420
 CTCACAT TTGCTTCTGA CATAGTTGTG TTGGGAGCTT GGATAGCTTG GGGGGGGGAC
 430 440 450 460 470 480
 AGCTCAGGGC TGCGATTTG CGCCAAACTT GACGGCAATC CTAGCGTGAA GGCTGGTAGG
 490 500 510 520 530 540
 ATTTTATCCC CGCTGCCATC ATGGTTCGAC CATTGAAC TG CATCGTCGCC GTGTCCCCAA
 550 560 570 580 590 600
 ATATGGGGAT TGGCAAGAAC GGAGACCTAC CCTGGCCTCC GCTCAGGAAC GAGTTCAAGT
 610 620 630 640 650 660
 ACTTCCAAAG AATGACCACA ACCTCTTCAG TGGAAGGTA ACAGAATCTG GTGATTATGG
 670 680 690 700 710 720
 GTAGGAAAC CTGGTTCTCC ATTCTGAGA AGAATCGACC TTAAAGGAC AGAATTAATA
 730 740 750 760 770 780
 TTCTCAG TAGAGAACTC AAAGAACAC CACGAGGGAC TCATTTCTT GCCAAAAGTT
 790 800 810 820 830 840
 TGGATGATGC CTTAACACTT ATTGAACAAAC CGGAATTGGC AAGTAAAGTA GACATGGTTT
 850 860 870 880 890 900
 GGATAGTCGG AGGCAGTTCT GTTTACCAAG AAGCCATGAA TCAACCAGGC CACCTCAGAC
 910 920 930 940 950 960
 TCTTGTGAC AAGGATCATG CAGGAATTG AAAGTGACAC GTTTTCCCA GAAATTGATT
 970 980 990 1000 1010 1020
 TGGGGAAATA TAAACTTCTC CCAGAACACC CAGGCGTCCT CTCTGAGGTC CAGGAGGAAA
 1030 1040 1050 1060 1070 1080
 AAGGCATCAA GTATAAGTTT GAAGTCTACG AGAAGAAAGA CTAACAGGAA GATGCTTCA
 1090 1100 1110 1120 1130 1140
 AGTTCTCTGC TCCCCTCCCT AAGCTATGCA TTTTATAAG ACCATGGGAC TTTGCTGGC
 1150 1160 1170 1180 1190 1200
 TTAGATCAG CCTCGACTGT GCCTTCTAGT TGCCAGCCAT CTGTTGTTG CCCCTCCCCC
 1210 1220 1230 1240 1250 1260
 GTGCCCTCCT TGACCCCTGGA AGGTGCCACT CCCACTGTCC TTTCCTAATA AAATGAGGAA
 1270 1280 1290 1300 1310 1320
 ATTGCATCGC ATTGTCTGAG TAGGTGTAT TCTATTCTGG GGGGTGGGT GGGGCAGGAC

FIGURE 7

DNASIS
Desmond

10 / 51

1330 1340 1350 1360 1370 1380
 AGCAAGGGGG AGGATTGGGA AGACAATAGC AGGCATGCTG GGGATGCGGT GGGCTCTATG
 1390 1400 1410 1420 1430 1440
 GCTTCTGAGG CGGAAAGAAC CAGCTGGGC TCGAAGCGGC CGCCCATTT GCTGGTGGTC
 1450 1460 1470 1480 1490 1500
 AGATGCGGGAGA TGGCGTGGGA CGCGGCGGGG AGCGTCACAC TGAGGTTTTC CGCCAGACGC
 1510 1520 1530 1540 1550 1560
 CACTGCTGCC AGGCCTGAT GTGCCCGGCT TCTGACCATG CGGTCGCGTT CGGTTGCACT
 1570 1580 1590 1600 1610 1620
 ACGCGTACTG TGAGCCAGAG TTGCCCAGGCT CTCTCCGGCT GCGGTAGTT AGGCAGTTCA
 1630 1640 1650 1660 1670 1680
 ATCAACTGTGTT TACCTTGTGG AGCGACATCC AGAGGCACCTT CACCGCTTGC CAGCGGCTTA
 1690 1700 1710 1720 1730 1740
 ATCCAGCG CCACCATCCA GTGCAGGAGC TCGTTATCGC TATGACGGAA CAGGTATTGCG
 1750 1760 1770 1780 1790 1800
 CTGGTCACCTT CGATGGTTTG CCCGGATAAA CGGAACCTGGA AAAACTGCTG CTGGTGTGTTT
 1810 1820 1830 1840 1850 1860
 GCTTCCGTCA GCGCTGGATG CGGCGTGCAG TCGGCAAAGA CCAGACCGTT CATAACAGAAC
 1870 1880 1890 1900 1910 1920
 TGGCGATCGT TCGGCGTATC GCCAAAATCA CCGCCGTAAG CCGACCCACGG GTTGGCGTTT
 1930 1940 1950 1960 1970 1980
 TCATCATATT TAATCAGCGA CTGATCCACC CAGTCCCAGA CGAAGCCGCC CTGTAAACGG
 1990 2000 2010 2020 2030 2040
 GGATACTGAC GAAACGCCCTG CCAGTATTAA GCGAAACCGC CAAGACTGTT ACCCATCGCG
 2050 2060 2070 2080 2090 2100
 CGCGTATT CGCAAAGGAT CAGCGGGCGC GTCTCTCCAG GTAGCGAAAG CCATTTTTG
 2110 2120 2130 2140 2150 2160
 ATGGACCATT TCGGCACAGC CGGGAAAGGGC TGGTCTTCAT CCACCGCGCGC GTACATCGGG
 2170 2180 2190 2200 2210 2220
 CAAATAATAT CGGTGGCCGT GGTGTCGGCT CCGCCGCCCTT CATACTGCAC CGGGCGGGAA
 2230 2240 2250 2260 2270 2280
 GGATCGACAG ATTTGATCCA GCGATAACAGC GCGTCGTGAT TAGCGCCGTG GCCTGATTCA
 2290 2300 2310 2320 2330 2340
 TTCCCCAGCG ACCAGATGAT CACACTCGGG TGATTACGAT CGCGCTGCAC CATTGCGTT
 2350 2360 2370 2380 2390 2400
 ACGCGTTCGC TCATGCCGG TAGCCAGCGC GGATCATCGG TCAGACGATT CATTGGCAC
 2410 2420 2430 2440 2450 2460
 ATGCCGTGGG TTTCAATATT GGCTTCATCC ACCACATACA GGCGTAGCG GTCGCACAGC
 2470 2480 2490 2500 2510 2520
 GTGTACCAACA CGGGATGGTT CGGATAATGC GAACAGCGCA CGGCCTTAAA GTTGTCTGC
 2530 2540 2550 2560 2570 2580
 TTCATCAGCA GGATATCCTG CACCATCGTC TGCTCATCCA TGACCTGACC ATGCAGAGGA
 2590 2600 2610 2620 2630 2640

DNASIS
Desmond Park

11 / 51

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 2710 2720 2730 2740 2750 2760
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 2770 2780 2790 2800 2810 2820
 AGTTTCGGGT TTTCGACGTT CAGACGTAGT GTGACGCGAT CGGCATAACC ACCACGCTCA
 2830 2840 2850 2860 2870 2880
 TCGATAATTT CACCGCCGAA AGGCGCGGTG CCGCTGGCGA CCTGCCTTTC ACCCTGCCAT
 2890 2900 2910 2920 2930 2940
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 2950 2960 2970 2980 2990 3000
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 3070 3080 3090 3100 3110 3120
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 3130 3140 3150 3160 3170 3180
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 3250 3260 3270 3280 3290 3300
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DNASIS

Desmond

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 4210 4220 4230 4240 4250 4260
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 4570 4580 4590 4600 4610 4620
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 4630 4640 4650 4660 4670 4680
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DNASIS
Desmond

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 5470 5480 5490 5500 5510 5520
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 5650 5660 5670 5680 5690 5700
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 5950 5960 5970 5980 5990 6000
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 6010 6020 6030 6040 6050 6060
 AACTATGTTA ATTITATGA GGAGAAAAAT GAAAAGAGA AGGAATACGA AGAAGAAAGAC
 6070 6080 6090 6100 6110 6120
 GACAAGGCCTG CTAGTTATG TGAAAATAAA ATTATATTGT CGCAAATTAA CTGTGAATCA
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 6250 6260 6270 6280 6290 6300
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 6310 6320 6330 6340 6350 6360
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 6370 6380 6390 6400 6410 6420
 TTTTCACCGA AGTCATGCC AGTCCAGCGT TTTTGCAGCA GAAAAGCCGC CGACTTCGGT
 6430 6440 6450 6460 6470 6480
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 6490 6500 6510 6520 6530 6540

DNASIS
Desmond [REDACTED]

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 6970 6980 6990 7000 7010 7020
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 7030 7040 7050 7060 7070 7080
 CTGTACAGTT CTTTCGGCTT GTTGCCCCGT TCGAAACCAA TGCCCTAAAGA GAGGTTAAAG
 7090 7100 7110 7120 7130 7140
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 7150 7160 7170 7180 7190 7200
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 7210 7220 7230 7240 7250 7260
 GCGTGGTCGT GCACCATCAG CACGTTATCG AATCCTTTGC CACGCAAGTC CGCATCTTC
 7270 7280 7290 7300 7310 7320
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 7330 7340 7350 7360 7370 7380
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 7390 7400 7410 7420 7430 7440
 GCTGTGACGC ACAGTTCATC GAGATAACCT TCACCCGGTT GCCAGAGGTG CGGATTCAACC
 7450 7460 7470 7480 7490 7500
 ACTTGCAAAG TCCCCCTAGT GCCTTGTCCA GTTGCAACCA CCTGTTGATC CGCATCACGC
 7510 7520 7530 7540 7550 7560
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 7570 7580 7590 7600 7610 7620
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 7630 7640 7650 7660 7670 7680
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 7690 7700 7710 7720 7730 7740
 TTCTTGCCGT TTTCGTCGGT AATCACCATT CCCGGCGGGGA TAGTCTGCCA GTTCAGTTCG
 7750 7760 7770 7780 7790 7800
 TTGTTCACAC AAACGGGTGAT ACCCCTCGAC GGATTAAGA CTTCAAGCGG TCAACTATGA

DNASIS
Desmond

7810 7820 7830 7840 7850 7860
 AGAAAGTGTTC GTCTTCGTCC CAGTAAGCTA TGTCTCCAGA ATGTAGGCCAT CCATCCTTGT
 7870 7880 7890 7900 7910 7920
 CAATCAAGGC GTTGGTCGCT TCCGGATTGT TTACATAACC GGACATAATC ATAGGTCCCTC
 7930 7940 7950 7960 7970 7980
 TGACACATAA TTCGCCTCTC TGATTAACGC CCAGCGTTTT CCCGGTATCC AGATCCACAA
 7990 8000 8010 8020 8030 8040
 CCTTCGCTTC AAAAAATGGA ACAACTTAC CGACCGCGCC CGGTTTATCA TCCCCCTCGG
 8050 8060 8070 8080 8090 8100
 GTGTAATCAG AATAGCTGAT GTAGTCTCAG TGAGCCCATA TCCTTGTGCG ATCCCTGGAA
 8110 8120 8130 8140 8150 8160
 GATGGAAGCG TTTTGCACCC GCTTCCCCGA CTTCTTCGA AAGAGGTGCG CCCCCAGAAG
 8170 8180 8190 8200 8210 8220
 1TTTCGTG TAAATTAGAT AAATCGTATT TGTCAATCAG AGTGCTTTG GCGAAGAACG
 8230 8240 8250 8260 8270 8280
 AAAATAGGGT TGGTACTAGC AACGCACTTT GAATTTTGTA ATCCTGAAGG GATCGTAAAA
 8290 8300 8310 8320 8330 8340
 ACAGCTCTTC TTCAAATCTA TACATTAAGA CGACTCGAAA TCCACATATC AAATATCCGA
 8350 8360 8370 8380 8390 8400
 GTGTAGTAAA CATTCCAAAA CCGTGATGGA ATGGAACAAC ACTTAAATC GCAGTATCCG
 8410 8420 8430 8440 8450 8460
 GAATGATTTG ATTGCCAAAA ATAGGATCTC TGGCATGCGA GAATCTGACG CAGGCAGTTC
 8470 8480 8490 8500 8510 8520
 TATGCGGAAG GGCCACACCC TTAGGTAACC CAGTAGATCC AGAGGAATTG TTTTGTACG
 8530 8540 8550 8560 8570 8580
 CAAAGGAC TCTGGTACAA AATCGTATTTC ATTAAAACCG GGAGGTAGAT GAGATGTGAC
 8590 8600 8610 8620 8630 8640
 GAACGTGTAC ATCGACTGAA ATCCCTGGTA ATCCGTTTTA GAATCCATGA TAATAATT
 8650 8660 8670 8680 8690 8700
 CTGGATTATT GGTAATTTTT TTTGCACGTT CAAAATTTTG TGCAACCCCT TTTTGGAAAC
 8710 8720 8730 8740 8750 8760
 AACACACTACG GTAGGCTGCG AAATGTTCAT ACTGTTGAGC AATTCACTT CATTATAAT
 8770 8780 8790 8800 8810 8820
 GTCGTTCGCG GGGCGCACTG CAACTCCGAT AAATAACGCG CCCAACACCG GCATAAAGAA
 8830 8840 8850 8860 8870 8880
 TTGAAGAGAG TTTTCACTGC ATACGACGAT TCTGTGATTG GTATTCAAGCC CATATCGTT
 8890 8900 8910 8920 8930 8940
 CATAGCTTCT GCCAACCGAA CGGACATTTC GAAGTATTCC GCGTACGTGA TGTTCACCTC
 8950 8960 8970 8980 8990 9000
 GATATGTGCA TCTGTAAAAG GAATTGTTCC AGGAACCAGG GCGTATCTCT TCATAGCCTT
 9010 9020 9030 9040 9050 9060
 ATGCAGTTGC TCTCCAGCGG TTCCATCCTC TAGCTTTGCT TCTCAATTTC TTATTGCA
 9070 9080 9090 9100 9110 9120
 AATGAGAAAA AAAGGAAAAT TAATTTAAC ACCAATTCAAG TAGTTGATTG AGCAAATGCG

DNASIS
Desmond

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9130 9140 9150 9160 9170 9180
 TTGCCAAAAA GGATGCTTA GAGACAGTGT TCTCTGCACA GATAAGGACA AACATTATTC
 9190 9200 9210 9220 9230 9240
 AGAGGGAGTA CCCAGAGCTG AGACTCCTAA GCCAGTGAGT GGCACAGCAT CCAGGGAGAA
 9250 9260 9270 9280 9290 9300
 ATATGCTTGT CATCACCGAA GCCTGATTCC GTAGAGCCAC ACCCTGGTAA GGGCCAATCT
 9310 9320 9330 9340 9350 9360
 GCTCACACAG GATAGAGAGG GCAGGAGCCA GGGCAGAGCA TATAAGGTGA GGTAGGATCA
 9370 9380 9390 9400 9410 9420
 GTTGCTCCTC ACATTTGCTT CTGACATAGT TGTGTTGGGA GCTTGGATCG ATCCACCATG
 9430 9440 9450 9460 9470 9480
 GGCTTCAATA CCCTGATTGA CTGGAACAGC TGTAGCCCTG AACAGCAGCG TGCGCTGCTG
 9490 9500 9510 9520 9530 9540
 ACGTCCGG CGATTTCCGC CTCTGACAGT ATTACCCGGA CGGTAGCGA TATTTGGAT
 9550 9560 9570 9580 9590 9600
 AATGTAAAAA CGCGCGGTGA CGATGCCCTG CGTGAATACA GCGCTAAATT TGATAAAACA
 9610 9620 9630 9640 9650 9660
 GAAGTGACAG CGCTACGCGT CACCCCTGAA GAGATGCCCG CGGCCGGCGC GCGTCTGAGC
 9670 9680 9690 9700 9710 9720
 GACGAATTAA AACAGGGCAT GACCGCTGCC GTCAAAAATA TTGAAACGTT CCATTCGCG
 9730 9740 9750 9760 9770 9780
 CAGACGCTAC CGCCTGTAGA TGTGGAAACC CAGCCAGGCG TGCGTTGCCA GCAGGTTACG
 9790 9800 9810 9820 9830 9840
 CGTCCCGTCT CGTCTGTCGG TCTGTATATT CCCGGCGGCT CGGCTCCGCT CTTCTAACG
 9850 9860 9870 9880 9890 9900
 CTGATGC TGGCGACGCC GGCGCGCATT GCGGGATGCC AGAAGGTGGT TCTGTGCTG
 9910 9920 9930 9940 9950 9960
 CCGCCGCCCA TCGCTGATGA AATCCTCTAT GCGGGCGAAC TGTGTGGCGT GCAGGAAATC
 9970 9980 9990 10000 10010 10020
 TTTAACGTGCG GCGGCGCGCA GGCGATTGCC GCTCTGGCCT TCGGCAGCGA GTCCGTACCG
 10030 10040 10050 10060 10070 10080
 AAAGTGGATA AAATTTTGG CCCCCGGCAAC GCCTTTGTAA CGAAGCCAA ACGTCAAGGTC
 10090 10100 10110 10120 10130 10140
 AGCCAGCGTC TCGACGGCGC GGCTATCGAT ATGCCAGCCG GGCGTCTGA AGTACTGGTG
 10150 10160 10170 10180 10190 10200
 ATCGCAGACA GCGGGCGAAC ACCGGATTC GTCGCTCTG ACCTGCTCTC CCAGGCTGAG
 10210 10220 10230 10240 10250 10260
 CACGGCCCGG ATTCCCAGGT GATCCTGCTG ACGCCTGATG CTGACATTGC CCGCAAGGTG
 10270 10280 10290 10300 10310 10320
 GCGGAGGCAG TAGAACGTCA ACTGGCGGAA CTGCCCGCG CGGACACCGC CCGGCAGGCC
 10330 10340 10350 10360 10370 10380
 CTGAGCGCCA GTCGTCTGAT TGTGACCAAA GATTTAGCGC AGTGCCTCGC CATCTCTAAT
 10390 10400 10410 10420 10430 10440

DNASIS
Desmond Park

CAGTATGGGC CGGAACACTT AATCATCCAG ACGCGCAATG CGCGCGATTG GGTGGATGCG
 10450 10460 10470 10480 10490 10500
 ATTACCAGCG CAGGCTCGGT ATTTCTCGGC GACTGGTCGC CGGAATCCGC CGGTGATTAC
 10510 10520 10530 10540 10550 10560
 GCTTCCGAA CCAACCATGT TTTACCGACC TATGGCTATA CTGCTACCTG TTCCAGCCTT
 10570 10580 10590 10600 10610 10620
 GGGTTAGCGG ATTTCCAGAA ACGGATGACC GTTCAGGAAC TGTGAAAGC GGGCTTTTCC
 10630 10640 10650 10660 10670 10680
 GCTCTGGCAT CAACCATTGA AACATTGGCG GC GG CAGAAC GTCTGACCGC CCATAAAAAT
 10690 10700 10710 10720 10730 10740
 GCCGTGACCC TGCGCGTAAA CGCCCTCAAG GAGCAAGCAT GAGCACTGAA AACACTCTCA
 10750 10760 10770 10780 10790 10800
 CGCTCGCTGA CTTAGCCC GT GAAATGTCC GCAACCTGGA GATCCAGACA TGGATAAGAT
 10810 10820 10830 10840 10850 10860
 ACATTGATGA GT TGGACAA ACCACAAC TA GAATGCAGTG AAAAATG C TTTATTTGTG
 10870 10880 10890 10900 10910 10920
 AAATTTGTGA TGCTATTGCT TTATTTGTA CCATTATAAG CTGCAATAAA CAAGTTAAC
 10930 10940 10950 10960 10970 10980
 ACAACAATTG CATTCA TTTT ATGTTTCAGG TTCAGGGGG A GGTGTGGGAG GTTTTTTAAA
 10990 11000 11010 11020 11030 11040
 GCAAGTAAA CCTCTACAAA TGTGGTATGG CTGATTATGA TCTCTAGGGC CGGCCCTCGA
 11050 11060 11070 11080 11090 11100
 CGGCGCGCCT GGGCGCTACT AACTCTCTCC TCCCTCCTT TTCCCTGCAGG CTCAAGGC
 11110 11120 11130 11140 11150 11160
 GCATGCCCGA CGGCGAGGAT CTCGTCGTGA CCCATGGCGA TGCCCTGCTTG CCGAATATCA
 11170 11180 11190 11200 11210 11220
 TGGTGGAAA TGGCCGCTT TCTGGATTCA TCGACTGTGG CCGGCTGGT GTGGCGGACC
 11230 11240 11250 11260 11270 11280
 GCTATCAGGA CATAGCGTTG GCTACCCGTG ATATTGCTGA AGAGCTTGGC GGCAGATGGG
 11290 11300 11310 11320 11330 11340
 CTGACCGCTT CCTCGTGCTT TACGGTATCG CCCCTCCGA TTCGCAGCGC ATGCCCTCT
 11350 11360 11370 11380 11390 11400
 ATCGCCCTCT TGACGAGTTC TTCTGAGCGG GACTCTGGGG TTCGAAATGA CCGACCAAGC
 11410 11420 11430 11440 11450 11460
 GACGCCAAC CTGCCATCAC GAGATTCGA TTCCACCGCC GCCTTCTATG AAAGGTTGGG
 11470 11480 11490 11500 11510 11520
 CTTCGGAATC GT TCCGGGG ACGCCGGCTG GATGATCCTC CAGCGCGGGG ATCTCATGCT
 11530 11540 11550 11560 11570 11580
 GGAGTTCTTC GCCCACCCA ACTTGTTAT TGCAAGCTTAT AATGGTTACA AATAAGCAA
 11590 11600 11610 11620 11630 11640
 TAGCATCACA AATTCACAA ATAAAGCATT TTTTCACTG CATTCTAGTT GTGGTTTGT
 11650 11660 11670 11680 11690 11700
 CAAACTCATC AATCTATCTT ATCATGTCTG GATCGCGGCC GGTCTCTCTC TAGCCCTAGG

DNASIS
Desmond Lark

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11710 11720 11730 11740 11750 11760
 TCTAGACTTG GCAGAACATA TCCATCGCGT CCGCCATCTC CAGCAGCCGC ACGCGGCCGA

 11770 11780 11790 11800 11810 11820
 TCTCGGGCAG CGTTGGGTCC TGGCCACGGG TGCGCATGAT CGTGCCTCTG TC GTTGAGGA

 11830 11840 11850 11860 11870 11880
 CCCGGCTAGG CTGGCGGGGT TGCCCTACTG GTTAGCAGAA TGAATCACCG ATACCGGAGC

 11890 11900 11910 11920 11930 11940
 GAACGTGAAG CGACTGCTGC TGCAAAACGT CTGCACCTG AGCAACAAACA TGAATGGTCT

 11950 11960 11970 11980 11990 12000
 TCGGTTTCGG TGTTTCGTA AGTCTGGAAA CGCGGAAGTC AGCGCCCTGC ACCATTATGT

 12010 12020 12030 12040 12050 12060
 TCCGGATCTG CATCGCAGGA TGCTGCTGGC TACCCCTGTGG AACACCTACA TCTGTATTAA

 12070 12080 12090 12100 12110 12120
 CGAAGCGCTG GCATTGACCC TGAGTGATTT TTCTCTGGTC CCGCCGCATC CATAACGCCA

 12130 12140 12150 12160 12170 12180
 GTTGTITACC CTCACAAACGT TCCAGTAACC GGGCATGTTC ATCATCAGTA ACCCGTATCG

 12190 12200 12210 12220 12230 12240
 TGAGCATCCT CTCTCGTTTC ATCGGTATCA TTACCCCCAT GAACAGAAAT CCCCCTTACA

 12250 12260 12270 12280 12290 12300
 CGGAGGCATC AGTGACCAAA CAGGAAAAAA CCGCCCTTAA CATGGCCCGC TTTATCAGAA

 12310 12320 12330 12340 12350 12360
 GCCAGACATT AACGCTTCTG GAGAAACTCA ACGAGCTGGA CGCGGATGAA CAGGCAGACA

 12370 12380 12390 12400 12410 12420
 TCTGTGAATC GCTTCACGAC CACCGCTGATG AGCTTTACCG CAGCTGCCCTC GCGCGTTTCG

 12430 12440 12450 12460 12470 12480
 GTGATGACGG TGAAAACCTC TGACACATGC AGCTCCCGA GACGGTCACA GCTTGTCTGT

 12490 12500 12510 12520 12530 12540
 AAGCGGATGC CGGGAGCAGA CAAGCCCCGTC AGGGCGCGTC AGCGGGTGT GGCGGGTGTG

 12550 12560 12570 12580 12590 12600
 GGGGCGCAGC CATGACCCAG TCACGTAGCG ATAGCGGAGT GTATACTGGC TTAACTATGC

 12610 12620 12630 12640 12650 12660
 GGCATCAGAG CAGATTGTAC TGAGAGTGC CCATATGCCG TGTGAAATAC CGCACAGATG

 12670 12680 12690 12700 12710 12720
 CGTAAGGAGA AAATACCGCA TCAGGCGCTC TTCCGCTTCC TCGCTCACTG ACTCGCTGCG

 12730 12740 12750 12760 12770 12780
 CTCGGTCGTT CGGCTGCGGC GAGCGGTATC AGCTCACTCA AAGGCGGTAA TACGGTTATC

 12790 12800 12810 12820 12830 12840
 CACAGAACATCA GGGGATAACG CAGGAAAGAA CATGTGAGCA AAAGGCCAGC AAAAGGCCAG

 12850 12860 12870 12880 12890 12900
 GAACCGTAAA AAGGCCGCGT TGCTGGCGTT TTCCCATAGG CTCCGCCCCC CTGACGAGCA

 12910 12920 12930 12940 12950 12960
 TCACAAAAAT CGACGCTCAA GTCAGAGGTG GCGAAACCCG ACAGGACTAT AAAGATAACCA

 12970 12980 12990 13000 13010 13020
 GGCGTTTCCC CCTGGAAGCT CCCTCGTGC CGTCTCTGTT CCGACCCCTGC CGCTTACCGG

DNASIS
Desmond

13030	13040	13050	13060	13070	13080
ATACCTGTCC GCCTTCTCC CTTGGGAAG CGTGGCGTT TCTCATAGCT CACGCTGTAG					
13090	13100	13110	13120	13130	13140
GTATCTCA GT TCGGTGTAGG TCGTTCGCTC CAAGCTGGGC TGTGTGCACG AACCCCCCGT					
13150	13160	13170	13180	13190	13200
TCAGCCGAC CGCTGCGCCT TATCCGGTAA CTATCGTCTT GAGTCCAACC CGGTAAGACA					
13210	13220	13230	13240	13250	13260
CGACTTATCG CCACTGGCAG CAGCCACTGG TAACAGGATT AGCAGAGCGA GGTATGTAGG					
13270	13280	13290	13300	13310	13320
CGGTGCTACA GAGTTCTTGA AGTGGTGCC TAAC TACGGC TACACTAGAA GGACAGTATT					
13330	13340	13350	13360	13370	13380
TGGTATCTGC GCTCTGCTGA AGCCAGTTAC CTTCGAAAAA AGAGTTGGTA GCTCTTGATC					
13390	13400	13410	13420	13430	13440
. CAAACAA ACCACCGCTG GTAGCGGTGG TTTTTTGTT TGCAAGCAGC AGATTACGCG					
13450	13460	13470	13480	13490	13500
CAGAAAAAAA GGATCTCAAG AAGATCCTTT GATCTTTCT ACGGGGTCTG ACGCTCAGTG					
13510	13520	13530	13540	13550	13560
GAACGAAAAC TCACGTTAAG GGATTTGGT CATGAGATT A TCAAAAGGA TCTTCACCTA					
13570	13580	13590	13600	13610	13620
GATCCTTTTA AATTAAAAAT GAAGTTTAA ATCAATCTAA AGTATATATG AGTAAACTTG					
13630	13640	13650	13660	13670	13680
GTCTGACAGT TACCAATGCT TAATCGTGA GGCACCTATC TCAGCGATCT GTCTATTTCG					
13690	13700	13710	13720	13730	13740
TTCATCCATA GTTGCTGAC TCCCCGTGCGT GTAGATAACT ACGATAACGGG AGGGCTTACC					
13750	13760	13770	13780	13790	13800
. CTGGCCCC AGTGCTGCAA TGATACCGCG AGACCCACGC TCACCGGCTC CAGATTATC					
13810	13820	13830	13840	13850	13860
AGCAATAAAC CAGCCAGCCG GAAGGGCCGA GCGCAGAAGT GGTCTGCAA CTTTATCCGC					
13870	13880	13890	13900	13910	13920
CTCCATCCAG TCTATTAATT GTTGCCGGGA AGCTAGAGTA AGTAGTTCGC CAGTTAATAG					
13930	13940	13950	13960	13970	13980
TTTGCACAC GTTGTGCCA TTGCTGCAGG CATCGTGGTG TCACGCTCGT CGTTTGGTAT					
13990	14000	14010	14020	14030	14040
GGCTTCATTC AGCTCCGGTT CCCAACGATC AAGGCCAGTT ACATGATCCC CCATGTTGTG					
14050	14060	14070	14080	14090	14100
CAAAAGCG GTTAGCTCCT TCGGTCTCC GATCGTTGTC AGAAGTAAGT TGGCCGCAGT					
14110	14120	14130	14140	14150	14160
GTTATCACTC ATGGTTATGG CAGCACTGCA TAATTCTCTT ACTGTCTGC CATCCGTAAG					
14170	14180	14190	14200	14210	14220
ATGCTTTCT GTGACTGGTG AGTACTCAAC CAAGTCATTC TGAGAATAGT GTATGCGCG					
14230	14240	14250	14260	14270	14280
ACCGAGTTGC TCTTGGCCCG CGTCAACACG GGATAATACC GCGCCACATA GCAGAACTTT					
14290	14300	14310	14320	14330	14340

DNASIS
Desmond rk

AAAAGTGCTC ATCATTGGAA AACGTTCTTC GGGGCAGAAA CTCTCAAGGA TCTTACCGCT

14350 14360 14370 14380 14390 14400
GTTGAGATCC AGTTGATGT AACCCACTCG TGCACCCAAC TGATCTTCAG CATCTTTAC

14410 14420 14430 14440 14450 14460
TTTCACCAGC GTTTCTGGGT GAGCAAAAC AGGAAGGCAA AATGCCGCAA AAAAGGGAAT

14470 14480 14490 14500 14510 14520
AAGGGCGACA CGGAAATGTT GAATACTCAT ACTCTTCCTT TTTCAATATT ATTGAAGCAT

14530 14540 14550 14560 14570 14580
TTATCAGGGT TATTGTCCTA TGAGCGGATA CATATTGAA TGTATTAGA AAAATAAACCA

14590 14600 14610 14620 14630 14640
AATAGGGGTT CCGCGCACAT TTCCCCGAAA AGTGCCACCT GACGTCTAAG AAACCATTAT

14650 14660 14670 14680 14690 14700
TATCATGACA TTAACCTATA AAAATAGGCG TATCACGAGG CCCTTTCGTC TTCAGAA..

DNASIS
Molly

FIGURE 8

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10 20 30 40 50 60
 TTAATTAAGG GGC GGAGAAT GGG CGGA ACT GGG CGG AGT AGGGG CGGG A TGG GC GG AGT
 70 80 90 100 110 120
 TAG GGG CGGG ACT ATGG TTG CTG ACT ATT GAG ATG CAT G CTT TGCA TAC TTCT GC CTGC
 130 140 150 160 170 180
 TGG GG AGC CT GGG GACT TTTC CAC ACCT GGT TG CTG ACT AA TTGAG ATG CA TG CT TTG CAT
 190 200 210 220 230 240
 ACT TT CTG C CT GCT GGG GAGC CT GGG GACT T CCAC ACCCT AACT GAC ACA CATT CCAC AGC
 250 260 270 280 290 300
 AATTA ATTCC CCTAG TTATT AATAG TAATC AATTAC GGGG TCATTAG TTTC ATAG CCC ATA
 310 320 330 340 350 360
 TAT GG AGT TC CGCG TTAC AT AAC TTAC GGT AA ATGG CCC G CCT GG CTG AC CGCC CAAC GA
 370 380 390 400 410 420
 CCG CCC A TTGAC GTCAA TAATGAC GTA TG TTCC ATA GTAA CGCC AA TAGG GACT TT
 430 440 450 460 470 480
 CCATTGAC GT CAATGG GT GG AGT ATT TAAG GTAA ACTG CC CACTT GG CAG TAC AT CAAGT
 490 500 510 520 530 540
 GTATCATATG CCAAGTAC GC CCC TATT GA CGT CAATG AC GGTAA ATGGC CGC CTGG CA
 550 560 570 580 590 600
 TTATGCC CAAG TAC CCT TAT GGG ACT T CC TACT TTG G CAG TAC AT CT ACG TATT AGT
 610 620 630 640 650 660
 CAT CG CT ATT ACC ATGG TGA TG CGG TTT TG GC AGT AC ATC AATGG CGT G GAT AG CG GTT
 670 680 690 700 710 720
 TG ACTC ACGG GG AT TT CCAA GT CT CCAC CC CATT GAC GT C AATGG AGT TG TT TTG AAG
 730 740 750 760 770 780
 TGG CGG GC CAG CT TT ATT TAAC GTG TT AC GTG AG TC AAT GTAC AC TAAC GAC AGT
 790 800 810 820 830 840
 GAT GAA AGAA ATAC AAA AGC GCATA AT ATT TTG AAC GAC G TCG AAC CCTT ATTAC AAA AC
 850 860 870 880 890 900
 AAA ACAC AAA CGA AT AT CGA CAA AGC TAGA TTG CTG CTAC AAG AT TTG GC AAG TT TTG TG
 910 920 930 940 950 960
 GCG TTG AGC G AAA AT CC ATT AG AT AGT CCA GCC AT CGG TT CGG AAAA ACA ACC TTG TT
 970 980 990 1000 1010 1020
 GAA ACTA ATC GAA AC CT ATT TTAC AA AT CT ATT GAG GATT TAAT ATT TAA ATT CAG AT AT
 1030 1040 1050 1060 1070 1080
 AAAG AC GCT G AAA AT CATT T GAT TT CG CT CTA AC AT ACC CTA AAGA TTATA AATT
 1090 1100 1110 1120 1130 1140
 AAT GAAT TAT TAAA ATAC AT CAG CA ACT AT ATT GAT AG AC AT TT CCAG TT GTG AT AT
 1150 1160 1170 1180 1190 1200
 TAG TT GT GC GT CT CATT AC AAT GG CT GT TT AT TT TAACA ACA AA CA ACT GCT CG CAG AC
 1210 1220 1230 1240 1250 1260
 AAT AGT ATAG AAA AGG GAGG TGA CT GT TT TTG TT AAC G GT CG TA CAA CATT TT GG AA
 1270 1280 1290 1300 1310 1320
 AGT TAT GT TA ATCC CGG TG CT GCT AAAA AT GGT GT AAT TG AACT AGA AAGA AG CT CG CT AC

DNASIS
Molly

1330 1340 1350 1360 1370 1380
 TATGCCGGCA ACATATTGTA CAAAACCGAC GATCCCAAAT TCATTGATTA TATAAATTTA

 1390 1400 1410 1420 1430 1440
 ATAATTAAAG CAACACACTC CGAAGAACTA CCAGAAAATA GCACTGTGT AAATTACAGA

 1450 1460 1470 1480 1490 1500
 AAAACTATGC GCAGCGGTAC TATACACCCC ATTAAAAAAG ACATATATAT TTATGACAAC

 1510 1520 1530 1540 1550 1560
 AAAAAATTAA CTCTATACGA TAGATACATA TATGGATACG ATAATAACTA TGTTAATTAA

 1570 1580 1590 1600 1610 1620
 TATGAGGAGA AAAATGAAAA AGAGAAGGAA TACGAAGAAG AAGACGACAA GGC GTCTAGT

 1630 1640 1650 1660 1670 1680
 TTATGTGAAA ATAAAATTAT ATTGTGCAA ATTAACGTG AATCATTTGA AAATGATTAA

 1690 1700 1710 1720 1730 1740
 AAATATTACC TCAGCGATTA TAACTACGCG TTTCAATTAA TAGATAATAC TACAAATGTT

 1750 1760 1770 1780 1790 1800
 CTTGTTGCGT TTGGTTTGTAA TCGTTAATAA AAAACAAATT TGACATTAT AATTGTTTAA

 1810 1820 1830 1840 1850 1860
 TTATTCAATA ATTACAAATA GGATTGAGAC CCTTGAGTT GCCAGCAAAC GGACAGAGCT

 1870 1880 1890 1900 1910 1920
 TGTGAGGAG AGTTGTTGAT TCATTGTTG CCTCCCTGCT GCGGTTTTTC ACCGAAGTTC

 1930 1940 1950 1960 1970 1980
 ATGCCAGTCC AGCGTTTTG CAGCAGAAAA GCCGCCGACT TCGGTTGCG GTCCCGAGTG

 1990 2000 2010 2020 2030 2040
 AAGATCCCTT TCTTGTACC GCCAACGCGC AATATGCCTT GCGAGGTCGC AAAATCGGCG

 2050 2060 2070 2080 2090 2100
 AAATTCATA CCTGTTCAACC GACGACGGCG CTGACGCGAT CAAAGACGCG GTGATAACATA

 2110 2120 2130 2140 2150 2160
 TCCAGCCATG CACACTGATA CTCTTCACTC CACATGTCGG TGTACATTGA GTGCAGCCCCG

 2170 2180 2190 2200 2210 2220
 GCTAACGTAT CCACGCCGTA TTCGGTGATG ATAATCGGCT GATGCAGTTT CTCCCTGCCAG

 2230 2240 2250 2260 2270 2280
 GCCAGAAGTT CTTTTCCAG TACCTTCTCT GCCGTTCCA AATCGCCGCT TTGGACATAC

 2290 2300 2310 2320 2330 2340
 CATCCGTAAT AACGGTTCAAG GCACAGCACA TCAAAGAGAT CGCTGATGGT ATCGGTGTGA

 2350 2360 2370 2380 2390 2400
 GCGTCGCGAGA ACATTACATT GACGCAGGTG ATCGGACGCG TCGGGTCGAG TTTACGCGTT

 2410 2420 2430 2440 2450 2460
 GCTTCCGCCA GTGGCGCGAA ATATTCCCGT GCACCTTGC GACGGGTATC CGGTTCGTTG

 2470 2480 2490 2500 2510 2520
 GCAATACTCC ACATCACCAC GCTTGGGTGG TTTTGTAC GCGCTATCAG CTCTTTAATC

 2530 2540 2550 2560 2570 2580
 GCCTGTAAGT GCGCTTGCTG AGTTTCCCCG TTGACTGCCT CTTCGCTGTA CAGTTCTTC

 2590 2600 2610 2620 2630 2640

DNASIS

Molly

GGCTTGTG CCGCTTCGAA ACCAATGCCT AAAGAGAGGT TAAAGCCGAC AGCAGCAGTT

2650 2660 2670 2680 2690 2700
TCATCAATCA CCACGATGCC ATGTTCATCT GCCCAGTCGA GCATCTCTTC AGCGTAAGGG

2710 2720 2730 2740 2750 2760
TAATGCGAGG TACGGTAGGA GTTGGCCCCA ATCCAGTCCA TTAATGCGTG GTCGTGCACC

2770 2780 2790 2800 2810 2820
ATCAGCACGT TATCGAATCC TTTGCCACGC AAGTCCGCAT CTTCATGACG ACCAAAGCCA

2830 2840 2850 2860 2870 2880
GTAAAGTAGA ACGGTTGTG GTTAATCAGG AACTGTTGC CTTTCACTGC CACTGACCGG

2890 2900 2910 2920 2930 2940
ATGCCGACGC GAAGCGGGTA GATATCACAC TCTGTCTGGC TTTGGCTGT GACGCACAGT

2950 2960 2970 2980 2990 3000
-ATAGAGAT AACCTTCACC CGGTTGCCAG AGGTGCGGAT TCACCACTTG CAAAGTCCCG

3010 3020 3030 3040 3050 3060
CTAGTGCCTT GTCCAGTTGC AACCACCTGT TGATCCGCAT CACGCAGTTC AACGCTGACA

3070 3080 3090 3100 3110 3120
TCACCAATTGG CCACCACTG CCAGTCAACA GACGGTGGT TACAGTCTTG CGCGACATGC

3130 3140 3150 3160 3170 3180
GTCACCAACGG TGATATCGTC CACCCAGGTG TTCGGCGTGG TGTAGAGCAT TACGCTGCAG

3190 3200 3210 3220 3230 3240
TGGATTCCGG CATAGTTAAA GAAATCATGG AAGTAAGACT GCTTTTCTT GCCGTTTTCG

3250 3260 3270 3280 3290 3300
TCGGTAATCA CCATTCGG CGGGATAGTC TGCCAGTTCA GTTCGTTGTT CACACAAACG

3310 3320 3330 3340 3350 3360
-TGATAACCCC TCGACGGATT AAAGACTTCA AGCGGTCAAC TATGAAGAAG TGTCGTCTT

3370 3380 3390 3400 3410 3420
CGTCCCAGTA AGCTATGTCT CCAGAATGTA GCCATCCATC CTTGTCAATC AAGGC GTTGG

3430 3440 3450 3460 3470 3480
TCGCTTCCGG ATTGTTTACA TAACCGGACA TAATCATAGG TCCTCTGACA CATAATTGCG

3490 3500 3510 3520 3530 3540
CTCTCTGATT AACGCCAGC GTTTCCGG TATCCAGATC CACAACCTTC GCTTCAAAAA

3550 3560 3570 3580 3590 3600
ATGGAACAAAC TTTACCGACC GCGCCCGTT TATCATCCCC CTGGGTGTA ATCAGAATAG

3610 3620 3630 3640 3650 3660
CTGATGTAGT CTCAGTGAGC CCATATCCTT GTCGTATCCC TGGAAGATGG AAGCGTTTGG

3670 3680 3690 3700 3710 3720
CAACCGCTTC CCCGACTTCT TTGAAAGAG GTGCGCCCCC AGAAGCAATT TCGTGTAAAT

3730 3740 3750 3760 3770 3780
TAGATAAACATC GTATTTGTCA ATCAGAGTGC TTTGGCGAA GAATGAAAAT AGGGTTGGTA

3790 3800 3810 3820 3830 3840
CTAGCAACGC ACTTTGAATT TTGTAATCCT GAAGGGATCG TAAAAACAGC TCTTCTCAA

3850 3860 3870 3880 3890 3900
ATCTATACAT TAAGACGACT CGAAATCCAC ATATCAAATA TCCGAGTGTAA GTAAACATTC

DNASIS

Molly

3910 3920 3930 3940 3950 3960
 CAAAACGTG ATGGAATGGA ACAACACTTA AAATCGCA GT ATCCGGAATG ATTGATTGC
 3970 3980 3990 4000 4010 4020
 CAAAAATAGG ATCTCTGGCA TGCGAGAAC TGACGCAGGC AGTTCTATGC GGAAGGGCCA
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 CACCCCTTAGG TAACCCAGTA GATCCAGAGG AATTGTTTG TCACGATCAA AGGACTCTGG
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 TACAAAATCG TATTCACTAA AACCGGGAGG TAGATGAGAT GTGACGAACG TGTACATCGA
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 CTGAAATCCC TGGTAATCCG TTTTACAATC CATGATAATA ATTTCTGGA TTATTGGTAA
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 TTTTTTTGCA ACGTTCAAA TTTTTGCAA CCCCTTTTG GAAACAAACA CTACGGTAGG
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 TCGAAATG TTCATACTGT TGAGCAATTG ACGTTCA TTAAATGTCGT TCGCGGGCGC
 4330 4340 4350 4360 4370 4380
 AACTGCAACT CCGATAAATA ACGCGCCAA CACCGGCATA AAGAATTGAA GAGAGTTTC
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 ACTGCATAAG ACGATTCTGT GATTTGTATT CAGCCCATAT CGTTTCATAG CTTCTGCCAA
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DNASIS
Molly

5230 5240 5250 5260 5270 5280
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 5290 5300 5310 5320 5330 5340
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 GTAAACGCC CCAAGGAGCA AGCATGAGGC ACTGAAAACA CTCTCAGCGT CGCTGACTTA
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 6490 6500 6510 6520 6530 6540

DNASIS
Molly L

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 6670 6680 6690 6700 6710 6720
 CCCAATGTCG AGCAGTGTGG TTTTGCAAGA GGAAGCAAAA AGCCTCTCCA CCCAGGCCTG
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 CAACAGACAA TCGGCTGCTC TGATGCCGCC GTGTTCCGGC TGTCAGCGA GGGCGCCCG
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DNASIS
Molly L.

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 GTGTACGGTG GGAGGTCTAT ATAAGCAGAG CTGGGTACGT GAACCGTCAG ATGCCCTGGA
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 GACGCCATCA CAGATCTCTC ACTATGGATT TTCAGGTGCA GATTATCAGC TTCTGCTAA
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 TCAGTGCTTC AGTCATAATG TCCAGAGGAC AAATTGTTCT CTCCCAGTCT CCAGCAATCC
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 TGTCTGCATC TCCAGGGGAG AAGGTACCAA TGACTTGCAG GGCCAGCTCA AGTGTAAAGTT
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DNASIS
Molly

9130	9140	9150	9160	9170	9180
GGACTTTCT	ACTTGGCAGT	ACATCTACGT	ATTAGTCATC	GCTATTACCA	TGGTGATGCG
9190	9200	9210	9220	9230	9240
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9250	9260	9270	9280	9290	9300
CCACCCCCATT	GACGTCAATG	GGAGTTTGT	TTGGCACCAA	AATCAACGGG	ACTTTCCAAA
9310	9320	9330	9340	9350	9360
ATGTCGTAAC	AACTCCGCC	CATTGACGCA	AATGGGCGGT	AGGC GTGTAC	GGTGGGAGGT
9370	9380	9390	9400	9410	9420
CTATATAAGC	AGAGCTGGGT	ACGT CCTCAC	ATTCAGTGAT	CAGCACTGAA	CACAGACCCG
9430	9440	9450	9460	9470	9480
TCGACATGGG	TTGGAGCCTC	ATCTTGCTCT	TCCTTGTGCG	TGTTGCTACG	CGTGTCTGT
9490	9500	9510	9520	9530	9540
CCCAGGTACA	ACTGCAGCAG	CCTGGGGCTG	AGCTGGTGA	GCCTGGGGCC	TCAGTGAAGA
9550	9560	9570	9580	9590	9600
TGT CCTGCAA	GGCTTCTGGC	TACACATTAA	CCAGTTACAA	TATGCACTGG	GTAAAACAGA
9610	9620	9630	9640	9650	9660
CACCTGGTCG	GGGCCTGGAA	TGGATTGGAG	CTATTTATCC	CGGAAATGGT	GATACTTCCT
9670	9680	9690	9700	9710	9720
ACAATCAGAA	GTTCAAAGGC	AAGGCCACAT	TGACTGCAGA	CAAATCCTCC	AGCACAGCCT
9730	9740	9750	9760	9770	9780
ACATGCAGCT	CAGCAGCCTG	ACATCTGAGG	ACTCTGCGGT	CTATTACTGT	GCAAGATCGA
9790	9800	9810	9820	9830	9840
CTTACTACGG	CGGTGACTGG	TACTTCAATG	TCTGGGGCGC	AGGGACCACG	GTCACCGTCT
9850	9860	9870	9880	9890	9900
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9910	9920	9930	9940	9950	9960
CTGGGGGCAC	AGCGGCCCTG	GGCTGCCTGG	TCAAGGACTA	CTTCCCCGAA	CCGGTGACGG
9970	9980	9990	10000	10010	10020
TGT CGTGGAA	CTCAGGCGCC	CTGACCAGCG	GCGTGCACAC	CTTCCC GGCT	GTCTTACAGT
10030	10040	10050	10060	10070	10080
CCTCAGGACT	CTACTCCCTC	AGCAGCGTGG	TGACCGTGCC	CTCCAGCAGC	TTGGGCACCC
10090	10100	10110	10120	10130	10140
AGACCTACAT	CTGCAACGTG	AATCACAAGC	CCAGCAACAC	CAAGGTGGAC	AAGAAAGCAG
10150	10160	10170	10180	10190	10200
AGCCCAAATC	TTGTGACAAA	ACTCACACAT	GCCCACCGTG	CCCAGCACCT	GAACTCCTGG
10210	10220	10230	10240	10250	10260
GGGGACCGTC	AGTCTTCTC	TTCCCCCAA	AACCCAAGGA	CACCCCTCATG	ATCTCCC GG
10270	10280	10290	10300	10310	10320
CCCCTGAGGT	CACATGC GTG	GTGGTGGACG	TGAGCCACGA	AGACCCGTAG	GTCAAGTTCA
10330	10340	10350	10360	10370	10380
ACTGGTACGT	GGACGGCGTG	GAGGTGCATA	ATGCCAAGAC	AAAGCCGGG	GAGGAGCAGT
10390	10400	10410	10420	10430	10440

DNASIS
Molly

ACAAACAGCAC GTACCGTGTG GTCAGCGTCC TCACCGTCT GCACCAGGAC TGGCTGAATG

10450 10460 10470 10480 10490 10500
GCAAGGAGTA CAAGTGCAAG GTCTCCAACA AAGCCCTCCC AGCCCCCATC GAGAAAACCA

10510 10520 10530 10540 10550 10560
TCTCCAAAGC CAAAGGGCAG CCCCAGAAC CACAGGTGTA CACCCCTGCC CCATCCCAGGG

10570 10580 10590 10600 10610 10620
ATGAGCTGAC CAAGAACCAAG GTCAGCCTGA CCTGCCTGGT CAAAGGCTTC TATCCCAGCG

10630 10640 10650 10660 10670 10680
ACATCGCCGT GGAGTGGGAG AGCAATGGGC AGCCGGAGAA CAACTACAAG ACCACGCCTC

10690 10700 10710 10720 10730 10740
CCGTGCTGGA CTCCGACGGC TCCTTCTTCC TCTACAGCAA GCTCACCGTG GACAAGAGCA

10750 10760 10770 10780 10790 10800
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10870 10880 10890 10900 10910 10920
TACCTAGACT GGATTGCGTGA CAACATGCGG CCGTGATATC TACGTATGAT CAGCCTCGAC

10930 10940 10950 10960 10970 10980
TGTGCCTTCT AGTTGCCAGC CATCTGTTGT TTGCCCTC CCCGTGCCTT CCTTGACCCCT

10990 11000 11010 11020 11030 11040
GGAAGGTGCC ACTCCCAGT TCCTTTCCTA ATAAAATGAG GAAATTGCAT CGCATTGTCT

11050 11060 11070 11080 11090 11100
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11110 11120 11130 11140 11150 11160
GAAGACAAT AGCAGGCATG CTGGGGATGC GGTGGGCTCT ATGGAACCAAG CTGGGGCTCG

11170 11180 11190 11200 11210 11220
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11230 11240 11250 11260 11270 11280
GAGGCAGCGC GGCTATCGTG GCTGGCCACG ACGGGCGTTC CTTGCGCAGC TGTGCTCGAC

11290 11300 11310 11320 11330 11340
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11350 11360 11370 11380 11390 11400
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11410 11420 11430 11440 11450 11460
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11470 11480 11490 11500 11510 11520
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11530 11540 11550 11560 11570 11580
CAGGGGGCTCG CGCCAGCCGA ACTGTTGCC AGGTAAGTGA GCTCCAATTTC AAGCTTCC

11590 11600 11610 11620 11630 11640
GGCGGGCCAG CTAGTAGCTT TGCTTCTCAA TTCTTATTT GCATAATGAG AAAAAAAGGA

11650 11660 11670 11680 11690 11700
AAATTAATTTC TAACACCAAT TCAGTAGTTG ATTGAGCAAA TGCCTTGCCA AAAAGGATGC

DNASIS
Molly

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11830	11840	11850	11860	11870	11880
CGAACGCTGA	TTCCGTAGAG	CCACACCCCTG	GTAAGGGCCA	ATCTGCTCAC	ACAGGATAGA
11890	11900	11910	11920	11930	11940
GAGGGCAGGA	GCCAGGGCAG	AGCATATAAG	GTGAGGTAGG	ATCAGTTGCT	CCTCACATT
11950	11960	11970	11980	11990	12000
GCTTCTGACA	TAGTTGTGTT	GGGAGCTTGG	ATAGCTTGGG	GGGGGGACAG	CTCAGGGCTG
12010	12020	12030	12040	12050	12060
CGATTTCGCG	CCAAACTTGA	CGGCAATCCT	AGCGTGAAGG	CTGGTAGGAT	TTTATCCCCG
12070	12080	12090	12100	12110	12120
GCCATCAT	GGTTCGACCA	TTGAAC TGCA	TCGTCGCCGT	GTCCAAAAT	ATGGGGATTG
12130	12140	12150	12160	12170	12180
GCAAGAACGG	AGACCTACCC	TGGCCTCCGC	TCAGGAACGA	GTTCAGTAC	TTCAAAGAA
12190	12200	12210	12220	12230	12240
TGACCACAAAC	CTCTTCAGTG	GAAGGTAAAC	AGAATCTGGT	GATTATGGGT	AGGAAAACCT
12250	12260	12270	12280	12290	12300
GGTTCTCCAT	TCCTGAGAAG	AATCGACCTT	TAAAGGACAG	AATTAATATA	GTTCAGTA
12310	12320	12330	12340	12350	12360
GAGAACTCAA	AGAACCAACCA	CGAGGAGCTC	ATTTCTTGC	CAAAAGTTG	GATGATGCCT
12370	12380	12390	12400	12410	12420
TAAGACTTAT	TGAACAAACCG	GAATTGGCAA	GTAAAGTAGA	CATGGTTGG	ATAGTCGGAG
12430	12440	12450	12460	12470	12480
.AGTTCTGT	TTACCAGGAA	GCCATGAATC	AACCAGGCCA	CCTCAGACTC	TTTGTGACAA
12490	12500	12510	12520	12530	12540
GGATCATGCA	GGAAATTGAA	AGTGACACGT	TTTCCCAGA	AATTGATTG	GGGAAATATA
12550	12560	12570	12580	12590	12600
AACTTCTCCC	AGAATACCCA	GGCGTCCTCT	CTGAGGTCCA	GGAGGAAAAA	GGCATCAAGT
12610	12620	12630	12640	12650	12660
ATAAGTTGA	AGTCTACGAG	AAGAAAGACT	AACAGGAAGA	TGCTTTCAAG	TTCTCTGCTC
12670	12680	12690	12700	12710	12720
CCCTCCTAAA	GCTATGCATT	TTTATAAGAC	CATGGGACTT	TTGCTGGCTT	TAGATCAGCC
12730	12740	12750	12760	12770	12780
TCGACTGTGC	CTTCTAGTTG	CCAGCCATCT	GTGTTTGCC	CCTCCCCGT	GCCTTCCTG
12790	12800	12810	12820	12830	12840
ACCCCTGGAAG	GTGCCACTCC	CACTGTCCTT	TCCTAATAAA	ATGAGGAAT	TGCATCGCAT
12850	12860	12870	12880	12890	12900
TGTCTGAGTA	GGTGTCAATT	TATTCTGGGG	GGTGGGGTGG	GGCAGGACAG	CAAGGGGGAG
12910	12920	12930	12940	12950	12960
GATTGGGAAG	ACAATAGCAG	GCATGCTGGG	GATGCGGTGG	GCTCTATGGC	TTCTGAGGCG
12970	12980	12990	13000	13010	13020
GAAAGAACCA	GCTGGGGCTC	GAAGCGGCCG	CCCATTTCGC	TGGTGGTCAG	ATGCGGGATG

DNASIS
Molly L.

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 GCGCTGATGT GCCCGGCTTC TGACCATGCCG GTCCGGTTCG GTTGCACTA GCCTACTGTG

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 AGCCAGAGTT GCCCGGCCTC CTCCGGCTGC GGTAGTTCAAG GCAGTTCAAT CAACTGTTA

 13210 13220 13230 13240 13250 13260
 CCTTGTTGGAG CGACATCCAG AGGCACCTCA CCGCTTGCCA GCGGCTTACCC ATCCAGCGCC

 13270 13280 13290 13300 13310 13320
 ACCATCCAGT GCAGGAGCTC GTTATCGCTA TGACGGAACA GGTATTGCT GTTCACTTCG

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 CAAAGGATCA GCGGGCGCGT CTCTCCAGGT AGCGAAAGCC ATTGTTTGAT GGACCATTT

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 GGCACAGCCG GGAAGGGCTG GTCTTCATCC ACGCGCGCGT ACATCGGGCA AATAATATCG

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 GGCCTGTCG TGTCGGCTCC GCGCCCTCA TACTGCACCG GGCAGGGAGG ATCGACAGAT

 13810 13820 13830 13840 13850 13860
 TTGATCCAGC GATACAGCGC GTCGTGATTA GCGCCGTGGC CTGATTCAATT CCCCAGCGAC

 13870 13880 13890 13900 13910 13920
 CAGATGATCA CACTCGGGTG ATTACGATCG CGCTGCACCA TTCGCGTTAC GCGTTCGCTC

 13930 13940 13950 13960 13970 13980
 ATCGCCGGTA GCCAGCGCGG ATCATCGGTC AGACGATTCA TTGGCACCAT GCGTGGGTT

 13990 14000 14010 14020 14030 14040
 TCAATATTGG CTTCATCCAC CACATACAGG CCGTAGCGGT CGCACAGCGT GTACCACAGC

 14050 14060 14070 14080 14090 14100
 GGATGGTTCG GATAATGCGA ACAGCGCACG GCGTTAAAGT TGTTCTGCTT CATCAGCGAGG

 14110 14120 14130 14140 14150 14160
 ATATCCTGCA CCATCGTCTG CTCATCCATG ACCTGACCAT GCAGAGGATG ATGCTCGTGA

 14170 14180 14190 14200 14210 14220
 CGGTTAACGC CTCGAATCAG CAACGGCTTG CCGTTCAAGA GCAGCAGACC ATTTCAATC

 14230 14240 14250 14260 14270 14280
 CGCACCTCGC GGAAACCGAC ATCGCAGGCT TCTGCTTCAA TCAGCGTGCC GTCGGCGGTG

 14290 14300 14310 14320 14330 14340

DNASIS
Molly L.

TGCAGTTCAA CCACCGCACG ATAGAGATTG GGGATTCGG CGCTCCACAG TTTCGGTTT
 14350 14360 14370 14380 14390 14400
 TCGACGTTCA GACGTAGTGT GACCGCATCG GCATAACCAC CACGCTCATC GATAATTCA
 14410 14420 14430 14440 14450 14460
 CCGCCGAAAG GCGCGGTGCC GCTGGCGACC TGCGTTTCAC CCTGCCATAA AGAAACTGTT
 14470 14480 14490 14500 14510 14520
 ACCCGTAGGT AGTCACGCAA CTCGCCGAC ATCTGAACCTT CAGCCTCCAG TACAGCGCGG
 14530 14540 14550 14560 14570 14580
 CTGAAATCAT CATTAAAGCG AGTGGCAACA TGGAATCGC TGATTTGTGT AGTCGGTTA
 14590 14600 14610 14620 14630 14640
 TGCAGCAACG AGACGTACG GAAAATGCCG CTCATCCGCC ACATATCCCTG ATCTTCCAGA
 14650 14660 14670 14680 14690 14700
 TAACTGCCGT CACTCCAGCG CAGCACCATC ACCGCGAGGC GGTTTTCTCC GGC CGTAAA
 14710 14720 14730 14740 14750 14760
 AATGCGCTCA GGTCAAATTC AGACGGCAAA CGACTGTCCG GGCCGTAACC GACCCAGCGC
 14770 14780 14790 14800 14810 14820
 CCGTTGCACC ACAGATGAAA CGCCGAGTTA ACGCCATCAA AAATAATTG CGTCTGGCCT
 14830 14840 14850 14860 14870 14880
 TCCTGTAGCC AGCTTCATC AACATTAAT GTGAGCGAGT AACAAACCGT CGGATTCTCC
 14890 14900 14910 14920 14930 14940
 GTGGGAACAA ACGGCGGATT GACCGTAATG GGATAGGTGA CGTTGGTGTG GATGGGGCGA
 14950 14960 14970 14980 14990 15000
 TCGTAACCGT GCATCTGCCA GTTGAGGGG ACGACGACAG TATCGGCCTC AGGAAGATCG
 15010 15020 15030 15040 15050 15060
 CACTCCAGCC AGCTTCGGG CACCGCTTCT GGTGCCGGAA ACCAGGCAA GCGCCATTG
 15070 15080 15090 15100 15110 15120
 CCATTCAAGGC TGCGCAACTG TTGGGAAGGG CGATCGGTGC GGGCCTCTTC GCTATTACGC
 15130 15140 15150 15160 15170 15180
 CAGCTGGCGA AAGGGGGATG TGCTGCAAGG CGATTAAGTT GGGTAACGCC AGGGTTTTCC
 15190 15200 15210 15220 15230 15240
 CAGTCACGAC GTTGTAAAAC GACTTAATCC GTCGAGGGGC TGCCCTGAAG CAGACGACCT
 15250 15260 15270 15280 15290 15300
 TCCGTTGTGC AGCCAGCGGC GCCTGCGCCG GTGCCCAACAA TCGTGCACGA ACAAACTAAA
 15310 15320 15330 15340 15350 15360
 CCAGAACAAA TTATACCGGC GGCACCGCCG CCACCACTT CTCCCGTGCCT TAACATTCCA
 15370 15380 15390 15400 15410 15420
 GCGCCCTCAC CACCAACCAC ACCATCGATG TCTGAATTGC CGCCCGCTCC ACCAATGCCG
 15430 15440 15450 15460 15470 15480
 ACGGAACCTC AACCCGCTGC ACCTTTAGAC GACAGACAA AATTGTTGGA AGCTATTAGA
 15490 15500 15510 15520 15530 15540
 AACGAAAAAA ATCGCACTCG TCTCAGACCG GTCAAACAA AAACGGCGCC CGAAACCAAGT
 15550 15560 15570 15580 15590 15600
 ACAATAGTTG AGGTGCCGAC TGTGTTGCCT AAAGAGACAT TTGAGCCTAA ACCGCCGTCT

DNASIS
Molly L

15610 15620 15630 15640 15650 15660
 GCATCACCGC CACCACTCC GCCTCCGCT CCGCCGCCAG CCCCACCTGC GCCTCCACCG
 15670 15680 15690 15700 15710 15720
 ATGGTAGATT TATCATCAGC TCCACCACCG CCGCCATTAG TAGATTTGCC GTCTGAAATG
 15730 15740 15750 15760 15770 15780
 TTACCAACCGC CTGCACCATC GCTTCTAAC GTGTTGTCTG AATTAAAATC GGGCACAGTT
 15790 15800 15810 15820 15830 15840
 AGATTGAAAC CCGCCCAAAA ACGCCCGCAA TCAGAAATAA TTCCAAAAAG CTCAACTACA
 15850 15860 15870 15880 15890 15900
 AATTTGATCG CGGACGTGTT AGCCGACACA ATTAATAGGC GTCGTGTGGC TATGGCAAAA
 15910 15920 15930 15940 15950 15960
 TCGTCTTCGG AAGCAACTTC TAACGACGAG GGTTGGGACG ACGACGATAA TCGGCCTAAT
 15970 15980 15990 16000 16010 16020
 AGCTAACACGCCCCATGT TAAATATGTC CAAGCTACTA GTGGTACCGC TTGGCAGAAC
 16030 16040 16050 16060 16070 16080
 ATATCCATCG CGTCCGCCAT CTCCAGCAGC CGCACGCGGC GCATCTCGGG CAGCGTTGGG
 16090 16100 16110 16120 16130 16140
 TCCTGGCCAC GGGTGCACAT GATCGTGTCTC CTGTCGTTGA GGACCCGGCT AGGCTGGCGG
 16150 16160 16170 16180 16190 16200
 GGTTGCCTTA CTGGTTAGCA GAATGAATCA CCGATAACGCG AGCGAACGTG AAGCGACTGC
 16210 16220 16230 16240 16250 16260
 TGCTGCAAAA CGTCTGCGAC CTGAGCAACA ACATGAATGG TCTTCGGTTT CCCTGTTTCG
 16270 16280 16290 16300 16310 16320
 TAAAGTCTGG AAACCGGAA GTCAGCGCCC TGCAACATTA TGTTCCGGAT CTGCATCGCA
 16330 16340 16350 16360 16370 16380
 GATGCTGCT GGCTACCCCTG TGGAACACCT ACATCTGTAT TAACGAAGCG CTGGCATTGA
 16390 16400 16410 16420 16430 16440
 CCCTGAGTGA TTTTCTCTG GTCCCGCCGC ATCCATACCG CCAGTTGTTT ACCCTCACAA
 16450 16460 16470 16480 16490 16500
 CGTTCCAGTA ACCGGGCATG TTCATCATCA GTAACCCGTA TCGTGAGCAT CCTCTCTCGT
 16510 16520 16530 16540 16550 16560
 TTCACTGGTA TCATTACCCC CATGAACAGA AATCCCCCTT ACACGGAGGC ATCAGTGACC
 16570 16580 16590 16600 16610 16620
 AACAGGAAA AAACCGCCCT TAACATGGCC CGCTTATCA GAAGCCAGAC ATTAACGCTT
 16630 16640 16650 16660 16670 16680
 CTGGAGAAAAC TCAACGAGCT GGACGCGGAT GAACAGGCAG ACATCTGTGA ATCGCTTCAC
 16690 16700 16710 16720 16730 16740
 GACCACGCTG ATGAGCTTTA CCGCAGCTGC CTCGCGCGTT TCGGTGATGA CGGTGAAAAC
 16750 16760 16770 16780 16790 16800
 CTCTGACACA TGCAGCTCCC GGAGACGGTC ACAGCTTGTC TGTAAGCGGA TGCCGGGAGC
 16810 16820 16830 16840 16850 16860
 AGACAAGCCC GTCAGGGCGC GTCAGCGGGT GTTGGCGGGT GTCGGGGCGC AGCCATGACC
 16870 16880 16890 16900 16910 16920
 CAGTCACGTA GCGATAGCGG AGTGTATACT GGCTTAACCA TGCGGCATCA GAGCAGATTG

DNASIS
Molly

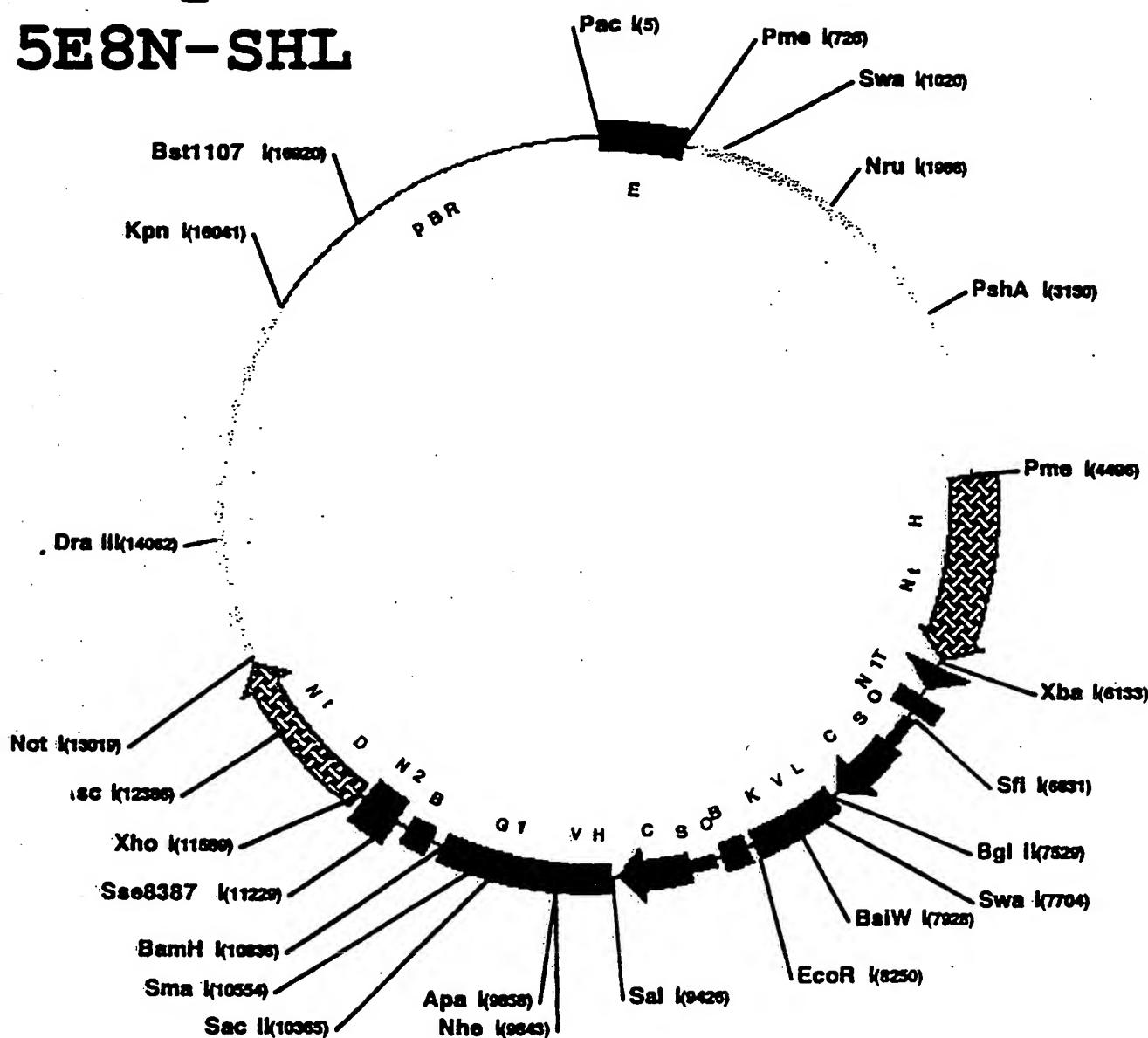
16930	16940	16950	16960	16970	16980
TACTGAGAGT GCACCATATG CGGTGTGAAA TACCGCACAG ATGCGTAAGG AGAAAATACC					
16990	17000	17010	17020	17030	17040
GCATCAGGCG CTCTCCGCT TCCTCGCTCA CTGACTCGCT GCGCTGGTC GTTCGGCTGC					
17050	17060	17070	17080	17090	17100
GGCGAGGGT ATCAGCTCAC TCAAAGGCGG TAATACGGTT ATCCACAGAA TCAGGGGATA					
17110	17120	17130	17140	17150	17160
ACGCAGGAAA GAACATGTGA GCAAAAGGCC AGCAAAAGGC CAGGAACCGT AAAAAGGCCG					
17170	17180	17190	17200	17210	17220
CGTTGCTGGC GTTTTCCAT AGGCTCCGCC CCCCTGACGA GCATCACAAA AATCGACGCT					
17230	17240	17250	17260	17270	17280
CAAGTCAGAG GTGGCGAAC CCGACAGGAC TATAAAGATA CCAGGCGTTT CCCCCCTGGAA					
17290	17300	17310	17320	17330	17340
GCTCCCTCGT GCGCTCTCCT GTTCCGACCC TGCCGCTTAC CGGATACTG TCCGCCCTTC					
17350	17360	17370	17380	17390	17400
TCCCTTCGGG AAGCGTGGCG CTTCTCATA GCTCACGCTG TAGGTATCTC AGTCGGTGT					
17410	17420	17430	17440	17450	17460
AGGTCGTTCG CTCCAAGCTG GGCTGTGTGC ACGAACCCCC CGTTCAGCCC GACCGCTGCG					
17470	17480	17490	17500	17510	17520
CCTTATCCGG TAACTATCGT CTTGAGTCCA ACCCGGTAAG ACACGACTTA TCGCCACTGG					
17530	17540	17550	17560	17570	17580
CAGCAGCCAC TGGTAACAGG ATTAGCAGAG CGAGGTATGT AGGCGGTGCT ACAGAGTTCT					
17590	17600	17610	17620	17630	17640
TGAAGTGGTG GCCTAACTAC GGCTACACTA GAAGGACAGT ATTTGGTATC TGCCTCTGC					
17650	17660	17670	17680	17690	17700
TGAAGCCAGT TACCTTCGGA AAAAGAGTTG GTAGCTTTG ATCCGGCAA CAAACCACCG					
17710	17720	17730	17740	17750	17760
CTGGTAGCCGG TGGTTTTTTT GTTGCAAGC AGCAGATTAC GCGCAGAAAA AAAGGATCTC					
17770	17780	17790	17800	17810	17820
AAGAAGATCC TTTGATCTTT TCTACGGGGT CTGACGCTCA GTGGAACGAA AACTCACGTT					
17830	17840	17850	17860	17870	17880
AAGGGATTTT GGTCACTGAGA TTATCAAAAA GGATCTTCAC CTAGATCCTT TAAATTAAA					
17890	17900	17910	17920	17930	17940
AATGAAGTTT TAAATCAATC TAAAGTATAT ATGAGTAAAC TTGGTCTGAC AGTTACCAAT					
17950	17960	17970	17980	17990	18000
GCTTAATCAG TGAGGCACCT ATCTCAGCGA TCTGTCTATT TCGTTCATCC ATAGTTGCCT					
18010	18020	18030	18040	18050	18060
GACTCCCCGT CGTGTAGATA ACTACGATAC GGGAGGGCTT ACCATCTGGC CCCAGTGCTG					
18070	18080	18090	18100	18110	18120
CAATGATACC GCGAGACCA CGCTCACCGG CTCCAGATTT ATCAGCAATA AACCAAGCCAG					
18130	18140	18150	18160	18170	18180
CCGGAAGGGC CGAGCGAGA AGTGGTCCTG CAACTTTATC CGCCTCCATC CAGTCTATTA					
18190	18200	18210	18220	18230	18240

DNASIS
Molly Lark

ATTGTTGCCG GGAAGCTAGA GTAAGTAGTT CGCCAGTTAA TAGTTTGCAC AACGTTGTTG
18250 18260 18270 18280 18290 18300
CCATTGCTGC AGGCATCGTG GTGTCACGCT CGTCGTTGG TATGGCTTCA TTCAGCTCCG
18310 18320 18330 18340 18350 18360
GTTCCCCAACG ATCAAGGCAGA GTTACATGAT CCCCCATGTT GTGAAAAAA GCGGTTAGCT
18370 18380 18390 18400 18410 18420
CCTTCGGTCC TCCGATCGTT GTCAGAAGTA AGTTGGCCGC AGTGTATCA CTCATGGTTA
18430 18440 18450 18460 18470 18480
TGGCAGCACT GCATAATTCT CTTACTGTCA TGCCATCCGT AAGATGCTTT TCTGTGACTG
18490 18500 18510 18520 18530 18540
GTGAGTACTC AACCAAGTCA TTCTGAGAAT AGTGTATGCG GCGACCGAGT TGCTCTGCC
18550 18560 18570 18580 18590 18600
GGCGTCAAC ACGGGATAAT ACCGCGCAC ATAGCAGAAC TTTAAAAGTG CTCATCATTG
18610 18620 18630 18640 18650 18660
GAAAACGTTTC TTGGGGCGA AAACTCTCAA GGATCTTACC GCTGTTGAGA TCCAGTTCGA
18670 18680 18690 18700 18710 18720
TGTAACCCAC TCGTGCACCC AACTGATCTT CAGCATCTT TACTTTCACC AGCGTTCTG
18730 18740 18750 18760 18770 18780
GGTGAGCAAA AACAGGAAGG CAAATGCCG CAAAAAAGGG AATAAGGGCG ACACGGAAAT
18790 18800 18810 18820 18830 18840
GTTGAATACT CATACTCTTC CTTTTCAAT ATTATTGAAG CATTTATCAG GTTTATTGTC
18850 18860 18870 18880 18890 18900
TCATGAGCGG ATACATATTT GAATGTATTT AGAAAAATAA ACAAAATAGGG GTTCCGCGCA
18910 18920 18930 18940 18950 18960
-TTTCCCCG AAAAGTCCA CCTGACGTCT AAGAAAACCAT TATTATCATG ACATTAACCT
18970 18980 18990 19000 19010 19020
ATAAAAATAG GCGTATCACG AGGCCCTTC GTCTTCAAGA A.....

Mandy + 5E8N-SHL

FIGURE 9



Nt D = Inactive Dihydrofolate reductase
 E = CMV and SV40 enhancers

Nt H = Inactive *Salmonella* Histidinol Dehydrogenase

T = Herpes Simplex thymidine kinase promoter and polyoma enhancer

C = Cytomegalovirus promoter/enhancer B = Bovine growth hormone polyadenylation

Nt I = Neomycin phosphotransferase exon 1 M2 = Neomycin phosphotransferase exon 2

K = Human kappa constant G1 = Human Gamma 1 constant

VL = Variable light chain anti-CD23 primate 5E8 and leader

VH = Variable heavy chain anti-CD23 primate 5E8N- and leader

SO = SV40 Origin of replication

Mandy cut Xba I and ligated to Xba I Xba I fragment from XKG1+CD23 5E8N-SHL

Map by Mitchell Reff

Constructed by Karen McLachlan

08/26/97

19,035 bp

FIGURE 10

DNASIS
Mandy E8N-SHL

10 20 30 40 50 60
 TTAATTAAGG GGC GGAGAAT GGG CGGA CT GGG CGG AGTT AGGGG C GGG A TG GGC GGG AGT
 70 80 90 100 110 120
 TAG GGG CGGG ACT ATGG TTG CT GACT AATT GAG ATG CATG CTT GCA TAC TT CTG CCT GC
 130 140 150 160 170 180
 TGG GAG CCT GGG ACT TTG CAC AC TG GT GACT AA TT GAG ATG CA TG CTT TG CAT
 190 200 210 220 230 240
 ACT TCT GCCT GCT GGG GAGC CT GGG ACT TT GCC AC ACC CTA AACT GAC ACA CATT CCAC AG
 250 260 270 280 290 300
 AATTA ATT CC CCT AG TT ATT AAT AG TAAT C AATT AC GGG G TC ATT AG TT C AT AG CCC ATA
 310 320 330 340 350 360
 TAT GGAG TT C CGC GTT ACAT AACT TAC GGT AA AT GG CCG CCT GG CTG AC CG CCC AAC GA
 370 380 390 400 410 420
 CCC CG CCC A TT GAC GT CAA TAAT GAC GT A TG TT CCC ATA GT AAC GCAA TAG GG ACT TT
 430 440 450 460 470 480
 CATT GAC GT CAAT GGG TGG AG TAT TTAC G TGAA ACT G CC ACT TGG CAG TAC AT CAAG T
 490 500 510 520 530 540
 GT AT CAT AT G CCA AGT AC GC CCC CT ATT GA CGT CA AT GAC G GT AA AT GG GC CC GCT GG CA
 550 560 570 580 590 600
 TT AT G C C C AG TAC AT GAC CT TAT GGG ACT T TG CT ACT TT GG CAG TAC AT CT ACG TATT AGT
 610 620 630 640 650 660
 CAT CG CT ATT ACC AT GGT GA TG CG GTT TTG GC AGT AC AT C AAT GGG CGT G GAT AG CG GTT
 670 680 690 700 710 720
 TG ACT CAC GG GG AT TT CCAA GT CT CCAC CC CATT GAC GT C AAT GGG AG TT TG TT TG AAG
 730 740 750 760 770 780
 GT TT AA AC AG CT TG G CCG GCC AG CT TT TA AC GT GT TT AC GT CG AG TCA AT TG TAC
 790 800 810 820 830 840
 ACT A AC GACA GT GAT GAA AG AA AT AC AAAA GCG CATA ATA TTT TGA AC GA CG TCG AAC CT
 850 860 870 880 890 900
 TT AT TAC AAAA ACA AA AC ACA AAC GA AT AC GACA AG CT GATT GCT GCT ACA AG AT TT G
 910 920 930 940 950 960
 GCA AG TT TG TGG CG TT TG AG CG AAA AT CCA TT AG AT AG TC CAG CC AT CGG TT CGG AAAA
 970 980 990 1000 1010 1020
 CA ACC CT TG TT TG GAA ACT AA TCG AA AC CT TA TTT AC AA AT CT ATT GAG GA TTT AAT AT TT
 1030 1040 1050 1060 1070 1080
 AA ATT CAG AT ATA AGA AC GC TG AAA AT CAT TT GAT TT TG CT CT A AC ATA CC ACC CT AAA
 1090 1100 1110 1120 1130 1140
 GATT AT AAT GAA ATT AT TAA AAT AC AT CAG CA ACT AT AT ATT GAT AG A CATT TCC
 1150 1160 1170 1180 1190 1200
 AG TT TG AT ATT AG TT GT GCG TCT CATT ACA AT GG CTG TT AT TT TAA CA AC AA AC AA
 1210 1220 1230 1240 1250 1260
 CT GCT CG CAG ACA AT AGT AT AG AAA AGG GA GGT GAA CT GT TT TT GT TT AA CG GTT CG TAC
 1270 1280 1290 1300 1310 1320
 AAC AT TT GG AA AG TT AT GT TA AT CC GG TG CT GCA AAAA AT GG GT TA AT TG AACT AGA A

DNASIS
Mandy 5E8N-SHL

1330	1340	1350	1360	1370	1380
GAAGCTGCCGT ACTATGCCGG CAACATATTG TACAAAACCG ACGATCCAA ATTCAATTGAT					
1390	1400	1410	1420	1430	1440
TATATAAATT TAATAATTAA AGCAACACAC TCCGAAGAAC TACAGAAAA TAGCACTGTT					
1450	1460	1470	1480	1490	1500
GTAAATTACA GAAAAACTAT GCGCAGCGGT ACTATACACC CCATTAAGAA AGACATATAT					
1510	1520	1530	1540	1550	1560
ATTTATGACA ACAAAAAATT TACTCTATAC GATAGATACA TATATGGATA CGATAATAAC					
1570	1580	1590	1600	1610	1620
TATGTTAATT TTTATGAGGA GAAAAATGAA AAAGAGAAGG AATACGAAGA AGAAGACGAC					
1630	1640	1650	1660	1670	1680
AAGGCCTCTA GTTTATGTGA AAATAAAATT ATATTGTCGC AAATTAAC TGATCATTT					
1690	1700	1710	1720	1730	1740
GAAAATGATT TAAATATTA CCTCAGCGAT TATAACTACG CGTTTCAAT TATAGATAAT					
1750	1760	1770	1780	1790	1800
ACTACAAATG TTCTTGTGC GTTTGGTTTG TATCGTTAAT AAAAACAAA TTGACATT					
1810	1820	1830	1840	1850	1860
ATAATTGTTT TATTATCAA TAATTACAAA TAGGATTGAG ACCCTTGAG TTGCCAGCAA					
1870	1880	1890	1900	1910	1920
ACGGACAGAG CTTGTCGAGG AGAGTTGTTG ATTCAATTGTT TGCCCTCCCTG CTGCGGTTT					
1930	1940	1950	1960	1970	1980
TCACCGAAGT TCATGCCAGT CCAGCGTTT TGCAAGCAGAA AAGCCGCCGA CTTCGGTTT					
1990	2000	2010	2020	2030	2040
CGGTCGCGAG TGAAGATCCC TTCTTGTAA CCGCCAACGC GCAATATGCC TTGCGAGGTC					
2050	2060	2070	2080	2090	2100
GCAAAATCGG CGAAATTCCA TACCTGTTCA CCGACGACGG CGCTGACGCG ATCAAAGACG					
2110	2120	2130	2140	2150	2160
CGGTGATACA TATCCAGCCA TGCACACTGA TACTCTTCAC TCCACATGTC GGTGTACATT					
2170	2180	2190	2200	2210	2220
GAGTGCAGCC CGGCTAACGT ATCCACGCCG TATTCGGTGA TGATAATCGG CTGATGCAGT					
2230	2240	2250	2260	2270	2280
TTCTCCTGCC AGGCCAGAAG TTCTTTTCC AGTACCTTCT CTGCCGTTTC CAAATGCCG					
2290	2300	2310	2320	2330	2340
CTTGGACAT ACCATCCGTA ATAACGGTTC AGGCACAGCA CATCAAAGAG ATCGCTGATG					
2350	2360	2370	2380	2390	2400
GTATCGGTGT GAGCGTCGCA GAACATTACA TTGACCGAGG TGATCGGACG CGTCGGGTCG					
2410	2420	2430	2440	2450	2460
AGTTTACGCG TTGCTCCGC CAGTGGCGCG AAATATTCCC GTGCACCTTG CGGACGGGTA					
2470	2480	2490	2500	2510	2520
TCCGGTTCGT TGGCAATACT CCACATCACC ACGCTGGGT GGTTTTGTC ACGCGCTATC					
2530	2540	2550	2560	2570	2580
AGCTTTAA TCGCCTGTAA GTGCGCTTGC TGAGTTCCC CGTTGACTGC CTCTCGCTG					
2590	2600	2610	2620	2630	2640

DNASIS
Mandy + 5E8N-SHL

TACAGTTCTT TCGGCTTGT GCCCCGTTCG AAACCAATGC CTAAAGAGAG GTTAAAGCCG
 2650 2660 2670 2680 2690 2700
 ACAGCAGCAG TTTCATCAAT CACCACGATG CCATGTTCAT CTGCCAGTC GAGCATCTCT
 2710 2720 2730 2740 2750 2760
 TCAGCGTAAG GGTAATGCGA GGTACGGTAG GAGTTGGCCC CAATCCAGTC CATTAATGCG
 2770 2780 2790 2800 2810 2820
 TGGTCGTGCA CCATCAGCAC GTTATCGAAT CCTTGCCAC GCAAGTCCGC ATCTTCATGA
 2830 2840 2850 2860 2870 2880
 CGACCAAAGC CAGTAAAGTA GAACGGTTTG TGGTTAATCA GGAACTGTTG GCCCTTCACT
 2890 2900 2910 2920 2930 2940
 GCCACTGACC GGATGCCGAC GCGAAGCGGG TAGATATCAC ACTCTGTCG GCTTTGGCT
 2950 2960 2970 2980 2990 3000
 TGACGCACA GTTCATAGAG ATAACCTTCA CCCGGTTGCC AGAGGTGCGG ATTCAACACT
 3010 3020 3030 3040 3050 3060
 TGCAAAGTCC CGCTAGTGCC TTGTCCAGTT GCAACCACCT GTTGATCCGC ATCACCGAGT
 3070 3080 3090 3100 3110 3120
 TCAACGCTGA CATCACCAATT GGCCACCAACC TGCCAGTCAA CAGACGCGTG GTTACAGTCT
 3130 3140 3150 3160 3170 3180
 TGCACGACAT GCGTCACCAAC GGTGATATCG TCCACCCAGG TGTTGGCGT GGTGTAGAGC
 3190 3200 3210 3220 3230 3240
 ATTACGCTGC GATGGATTCC GGCATAGTTA AAGAAATCAT GGAAGTAAGA CTGCTTTTC
 3250 3260 3270 3280 3290 3300
 TTGCCGTTTT CGTCGGTAAT CACCATTCCC GGCGGGATAG TCTGCCAGTT CAGTTCGTTG
 3310 3320 3330 3340 3350 3360
 TCACACAAA CGGTGATACC CCTCGACGGA TTAAAGACTT CAAGCGGTCA ACTATGAAGA
 3370 3380 3390 3400 3410 3420
 AGTGTTCGTC TTCTCCCAG TAAGCTATGT CTCCAGAATG TAGCCATCCA TCCTTGTCAA
 3430 3440 3450 3460 3470 3480
 TCAAGGCAGTT GGTCGCTTCC GGATTGTTTA CATAACCGGA CATAATCATA GGTCCCTCTGA
 3490 3500 3510 3520 3530 3540
 CACATAATTG GCCTCTCTGA TTAACGCCA GCGTTTTCCC GGTATCCAGA TCCACAAACCT
 3550 3560 3570 3580 3590 3600
 TCGCTTCAAA AAATGGAACA ACTTTACCGA CCGCGCCCGG TTTATCATCC CCCTCGGGTG
 3610 3620 3630 3640 3650 3660
 TAATCAGAAT AGCTGATGTA GTCTCAGTGA GCCCATATCC TTGTGTTATC CCTGGAAAGAT
 3670 3680 3690 3700 3710 3720
 GGAAGCGTTT TGCAACCGCT TCCCCGACTT CTTCGAAAG AGGTGCGCCC CCAGAAGCAA
 3730 3740 3750 3760 3770 3780
 TTTCGTGTAAT TAGAGATAAA TCGTATTGTC CAATCAGAGT GCTTTGGCG AAGAATGAAA
 3790 3800 3810 3820 3830 3840
 ATAGGGTTGG TACTAGAAC GCACCTTGAA TTTTGTAAATC CTGAAGGGAT CGTAAAAAACAA
 3850 3860 3870 3880 3890 3900
 GCTCTTCTTC AAATCTATAC ATTAAGACGA CTCGAAATCC ACATATCAAATCAGTG

DNASIS
Mandy + S8N-SHL

3910	3920	3930	3940	3950	3960
TAGTAAACAT TCCAAAACCG TGATGGAATG GAACAACACT TAAAATCGCA GTATCCGGAA					
3970	3980	3990	4000	4010	4020
TGATTTGATT GCCAAAAATA GGATCTCTGG CATGCGAGAA TCTGACGCAG GCAGTTCTAT					
4030	4040	4050	4060	4070	4080
GCGGAAGGGC CACACCCCTTA GGTAAACCCAG TAGATCCAGA GGAATTGTTT TGTACAGATC					
4090	4100	4110	4120	4130	4140
AAAGGACTCT GGTACAAAAT CGTATTCTATT AAAACCGGGGA GGTAGATGAG ATGTGACGAA					
4150	4160	4170	4180	4190	4200
CGTGTACATC GACTGAAATC CCTGGTAATC CGTTTGTAGAA TCCATGATAA TAATTTTCTG					
4210	4220	4230	4240	4250	4260
GATTATTGGT AATTTTTTTT GCACGTTCAA AATTTTTTGC AACCCCTTTT TGAAACAAA					
4270	4280	4290	4300	4310	4320
.CTACGGTA GGCTGCGAAA TGTTCATACT GTTGAGCAAT TCACGTTCAT TATAATGTC					
4330	4340	4350	4360	4370	4380
GTTCGCGGGC GCAACTGCAA CTCCGATAAAA TAACCGCGCC AACACCGGCA TAAAGAATTG					
4390	4400	4410	4420	4430	4440
AAGAGAGTTT TCACTGCATA CGACGATTCT GTGATTTGTA TTCAGCCCCAT ATCGTTTCAT					
4450	4460	4470	4480	4490	4500
AGCTTCTGCC AACCGAACGG ACATTTGAA GTATTCGCG TACAGCCCCG CCGTTTAAAC					
4510	4520	4530	4540	4550	4560
GGCCGGGCTT CAATACCCCTG ATTGACTGGA ACAGCTGTAG CCCTGAACAG CAGCGTGC					
4570	4580	4590	4600	4610	4620
TGCTGACCGG TCCGGCGATT TCCGCCCTTG ACAGTATTAC CCGGACGGTC AGCGATATT					
4630	4640	4650	4660	4670	4680
.GATAATGT AAAACCGCGC GGTGACGATG CCCTGCGTGA ATACAGCGCT AAATTTGATA					
4690	4700	4710	4720	4730	4740
AAACAGAAGT GACAGCGCTA CGCGTCACCC CTGAAGAGAT CGCCGCCGCC GGCGCGCGTC					
4750	4760	4770	4780	4790	4800
TGAGCGACGA ATTAAAACAG GCGATGACCG CTGCCGTCAA AAATATTGAA ACgttccatt					
4810	4820	4830	4840	4850	4860
CCGCGCAGAC GCTACCGCCT GTAGATGTGG AAACCCAGCC AGGCCTGCGT TGCCAGCAGG					
4870	4880	4890	4900	4910	4920
TTACCGCGTCC CGTCTCGTCT GTCGGTCTGT ATATTCGGG CGGCTCGGCT CCGCTCTCT					
4930	4940	4950	4960	4970	4980
CAACGGTGTCT GATGCTGGCG ACGCCGGCGC GCATTGCGGG ATGCCAGAAG GTGGTTCTGT					
4990	5000	5010	5020	5030	5040
GCTCGCCGCC GCCCATCGCT GATGAAATCC TCTATCGGC GCAACTGTGT GGCCTGCGAGG					
5050	5060	5070	5080	5090	5100
AAATCTTTAA CGTCGGCGGC GCGCAGGGCA TTGCCGCTCT GGCCCTCGGC AGCGAGTCCG					
5110	5120	5130	5140	5150	5160
TACCGAAAGT GGATAAAATT TTTGGCCCCG GCAACGCCCT TGTAACCAGA GCAAACGTC					
5170	5180	5190	5200	5210	5220
AGGTCAAGCCA GCGTCTCGAC GCGCGGGCTA TCGATATGCC AGCCGGGCCG TCTGAAGTAC					

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5230 5240 5250 5260 5270 5280
 TGGTGATCGC AGACAGCGGC GCAACACCGG ATTCGTGCGC TTCTGACCTG CTCTCCAGG
 5290 5300 5310 5320 5330 5340
 CTGAGCACGG CCCGGATTCC CAGGTGATCC TGCTGACGCC TGATGCTGAC ATTGCCCGCA
 5350 5360 5370 5380 5390 5400
 AGGTGGCGGA GGCGGTAGAA CGTCAACTGG CGGAAC TGCC GCGCGCGGAC ACCGCCCCGC
 5410 5420 5430 5440 5450 5460
 AGGCCCTGAG CGCCAGTCGT CTGATTGTGA CCAAAGATT AGCGCAGTGC GTCGCCATCT
 5470 5480 5490 5500 5510 5520
 CTAATCAGTA TGGGCCGGAA CACTTAATCA TCCAGACGCC CAATGCCGC GATTTGGTGG
 5530 5540 5550 5560 5570 5580
 ATGCGATTAC CAGCGCAGGC TCGGTATTT TC GGCGACTG GTCGCCGGAA TCCGCCGGTG
 5590 5600 5610 5620 5630 5640
 ATTACGCTTC CGGAACCAAC CATGTTTAC CGACCTATGG CTATACTGCT ACCTGTTCCA
 5650 5660 5670 5680 5690 5700
 GCCTTGGGTT AGCGGATTC CAGAACCGGA TGACCGTTCA GGAACGTGCG AAAGCGGGCT
 5710 5720 5730 5740 5750 5760
 TTTCCGCTCT GGCATCAACC ATTGAAACAT TGGCGCGGC AGAACGTCTG ACCGCCATA
 5770 5780 5790 5800 5810 5820
 AAAATGCCGT GACCCCTGCC GTAAACGCC TCAAGGAGCA AGCATGAGCA CTGAAAACAC
 5830 5840 5850 5860 5870 5880
 TCTCAGCGTC GCTGACTTAG CCCGTGAAAA TGTCGCAAC CTGGAGATCC AGACATGGAT
 5890 5900 5910 5920 5930 5940
 ^AGATACATT GATGAGTTG GACAAACAC AACTAGAATG CAGTGA AAAA AATGCTTAT
 5950 5960 5970 5980 5990 6000
 TTGTGAAATT TGTGATGCTA TTGCTTTATT TGTAACCATT ATAAGCTGCA ATAACACAAGT
 6010 6020 6030 6040 6050 6060
 TAACAACAAC AATTGCATTC ATTTATGTT TCAGGTTCA GGGGAGGTGT GGGAGGTTTT
 6070 6080 6090 6100 6110 6120
 TTAAAGCAAG TAAAACCTCT ACAAAATGTGG TATGGCTGAT TATGATCTCT AGGGCCGGCC
 6130 6140 6150 6160 6170 6180
 CTCGACGGCG CGTCTAGAGC AGTGTGGTT TCAAGAGGAA GCAAAAGCC TCTCCACCCA
 6190 6200 6210 6220 6230 6240
 GGCCTGGAAT GTTCCACCC AATGTCGAGC AGTGTGGTT TGCAAGAGGA AGCAAAAGC
 6250 6260 6270 6280 6290 6300
 CTCTCCACCC AGGCCTGGAA TGTTCCACC CAATGTCGAG CAAACCCCGC CCAGCGTCTT
 6310 6320 6330 6340 6350 6360
 GTCATTGGCG AATTGGAACA CGCATATGCA GTCGGGCGG CGCGGTCCCA GGTCCACTTC
 6370 6380 6390 6400 6410 6420
 GCATATTAAG GTGGCGCGTG TGGCCTCGAA CACCGAGCGA CCCTGCAAGCC AATATGGGAT
 6430 6440 6450 6460 6470 6480
 CGGCCATTGA ACAAGATGGA TTGCACGCCAG GTTCTCCGGC CGCTTGGGTG GAGAGGCTAT
 6490 6500 6510 6520 6530 6540

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TCGGCTATGA CTGGGCACAA CAGACAATCG GCTGCTCTGA TGCCGCCGTG TTCCGGCTGT
 6550 6560 6570 6580 6590 6600
 CAGCGCAGGG GCGCCCGGTT CTCCCCGTCA AGACCGACCT GTCCGGTGCC CTGAATGAAC
 6610 6620 6630 6640 6650 6660
 TGCAGGTAAG TGCAGGCCGTC GATGGCCGAG GCGGCCCTCGG CCTCTGCATA AATAAAAAAA
 6670 6680 6690 6700 6710 6720
 ATTAGTCAGC CATGCATGGG GCGGAGAATG GGCAGGACTG GGCAGGAGTTA GGGCGGGAT
 6730 6740 6750 6760 6770 6780
 GGGCGGAGTT AGGGGCGGGA CTATGGTTGC TGACTAATTG AGATGCATGC TTTGCATACT
 6790 6800 6810 6820 6830 6840
 TCTGCCTGCT GGGGAGCCTG GGGACTTTC ACACCTGGTT GCTGACTAAT TGAGATGCAT
 6850 6860 6870 6880 6890 6900
 CCTTTCATA CTTCTGCCTG CTGGGGAGCC TGGGGACTTT CCACACCCCTA ACTGACACAC
 6910 6920 6930 6940 6950 6960
 ATTCCACAGA ATTAATTCCC CTAGTTATTA ATAGTAATCA ATTACGGGGT CATTAGTTCA
 6970 6980 6990 7000 7010 7020
 TAGCCCATAT ATGGAGTTCC GCGTTACATA ACTTACGGTA AATGGCCCCGC CTGGCTGACC
 7030 7040 7050 7060 7070 7080
 GCCCAACGAC CCCCGCCCAT TGACGTCAAT AATGACGTAT GTTCCCATAG TAACGCCAAT
 7090 7100 7110 7120 7130 7140
 AGGGACTTTC CATTGACGTC AATGGGTGGA GTATTTACGG TAAACTGCC ACTTGGCAGT
 7150 7160 7170 7180 7190 7200
 ACATCAAGTG TATCATAATGC CAAGTACGCC CCCTATTGAC GTCAATGACG GTAAATGGCC
 7210 7220 7230 7240 7250 7260
 CCTCTGGCAT TATGCCCACT ACATGACCTT ATGGGACTTT CCTACTTGGC AGTACATCTA
 7270 7280 7290 7300 7310 7320
 CGTATTAGTC ATCGCTATTA CCATGGTGAT GCGGTTTTGG CAGTACATCA ATGGCGTG
 7330 7340 7350 7360 7370 7380
 ATAGCGGTTT GACTCACGGG GATTTCCAAG TCTCCACCCC ATTGACGTCA ATGGGAGTTT
 7390 7400 7410 7420 7430 7440
 GTTTTGGCAC CAAAATCAAC GGGACTTTCC AAAATGTCGT AACAACTCCG CCCCATGAC
 7450 7460 7470 7480 7490 7500
 GCAAATGGGC GGTAGGCCTG TACGGTGGGA GGTCTATATA AGCAGAGCTG GGTACGTGAA
 7510 7520 7530 7540 7550 7560
 CCGTCAGATC GCCTGGAGAC GCCATCACAG ATCTCTCACC ATGGACATGA GGGTCCCCGC
 7570 7580 7590 7600 7610 7620
 TCAGCTCCTG GGGCTCCTTC TGCTCTGGCT CCCAGGTGCC AGATGTGACA TCCAGATGAC
 7630 7640 7650 7660 7670 7680
 CCAGTCTCCA TCTTCCCTGT CTGCATCTGT AGGGGACAGA GTCACCATCA CTTGCAGGGC
 7690 7700 7710 7720 7730 7740
 AAGTCAGGAC ATTAGGTATT ATTAAATTG GTATCAGCAG AAACCAAGGAA AAGCTCCTAA
 7750 7760 7770 7780 7790 7800
 GCTCCCTGATC TATGTTGCAT CCAGTTGCA AAGTGGGGTC CCATCAAGGT TCAGCGGCAG

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7810	7820	7830	7840	7850	7860
TGGATCTGGG ACAGAGTTCA CTCTCACCGT CAGCAGCTG CAGCCTGAAG ATTTTCGAC					
7870	7880	7890	7900	7910	7920
TTATTACTGT CTACAGGTTT ATAGTACCCC TCGGACGTT GCCTAAAGGG ACAAAGGTGGA					
7930	7940	7950	7960	7970	7980
AATCAAACGT ACGGTGGCTG CACCATCTGT CTTCATCTTC CCGCCATCTG ATGAGCAGTT					
7990	8000	8010	8020	8030	8040
GAAATCTGGA ACTGCCTCTG TTGTGTGCCT GCTGAATAAC TTCTATCCCA GAGAGGCCAA					
8050	8060	8070	8080	8090	8100
AGTACAGTGG AAGGTGGATA ACGCCCTCCA ATCGGGTAAC TCCCAGGAGA GTGTACAGA					
8110	8120	8130	8140	8150	8160
GCAGGACAGC AAGGACAGCA CCTACAGCCT CAGCAGCACC CTGACGCTGA GCAAAGCAGA					
8170	8180	8190	8200	8210	8220
TACGAGAAA CACAAAGTCT ACGCCTGCAG AGTCACCCAT CAGGGCCTGA GCTCGCCCGT					
8230	8240	8250	8260	8270	8280
CACAAAGAGC TTCAACAGGG GAGAGTGTG AATTCAAGATC CGTTAACGGT TACCAACTAC					
8290	8300	8310	8320	8330	8340
CTAGACTGGA TTCGTGACAA CATGCGGCCG TGATATCTAC GTATGATCAG CCTCGACTGT					
8350	8360	8370	8380	8390	8400
GCCTTCTAGT TGCCAGCCAT CTGTTGTTG CCCCTCCCCC GTGCCCTCT TGACCCCTGGA					
8410	8420	8430	8440	8450	8460
AGGTGCCACT CCCACTGTCC TTTCTTAATA AAATGAGGAA ATTGCATCGC ATTGTCTGAG					
8470	8480	8490	8500	8510	8520
TAGGTGTCAAT TCTATTCTGG GGGGTGGGGT GGGGCAGGAC AGCAAGGGGG AGGATTGGGA					
8530	8540	8550	8560	8570	8580
ACAATAGC AGGCATGCTG GGGATGCGGT GGGCTCTATG GCTTCTGAGG CGGAAAGAAC					
8590	8600	8610	8620	8630	8640
CAGCTGGGAC TAGTCGAAT TGGGGGGAGT TAGGGGGGGG ATGGGGGGAG TTAGGGGGCG					
8650	8660	8670	8680	8690	8700
GAECTATGGTT GCTGACTAAT TGAGATGCAT GCTTGCATA CTTCTGCCTG CTGGGGAGCC					
8710	8720	8730	8740	8750	8760
TGGGGACTTT CCACACCTGG TTGCTGACTA ATTGAGATGC ATGCTTTGCA TACTTCTGCC					
8770	8780	8790	8800	8810	8820
TGCTGGGGAG CCTGGGGACT TTCCACACCC TAACTGACAC ACATTCCACA GAATTAATTC					
8830	8840	8850	8860	8870	8880
CCCTAGTTAT TAATAGTAAT CAATTACGGG GTCATTAGTT CATAGCCCAT ATATGGAGTT					
8890	8900	8910	8920	8930	8940
CCGCCTTACA TAACTTACGG TAAATGGCCC GCCTGGCTGA CCGCCCAACG ACCCCCCCCC					
8950	8960	8970	8980	8990	9000
ATTGACGTCA ATAATGACGT ATGTTCCCAT AGTAACGCCA ATAGGGACTT TCCATTGACG					
9010	9020	9030	9040	9050	9060
TCAATGGGTG GAGTATTTAC GGTAAACTGC CCACTGGCA GTACATCAAG TGTATCATAT					
9070	9080	9090	9100	9110	9120
GCCAAGTACG CCCCCCTATTG ACGTCAATGA CGGTAAATGG CCGGCTGGC ATTATGCCCA					

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9130	9140	9150	9160	9170	9180
GTACATGACC	TTATGGGACT	TTCCTACTTG	GCACTACATC	TACGTATTAG	TCATCGCTGT
9190	9200	9210	9220	9230	9240
TACCATGGTG	ATGCGGTTT	GGCAGTACAT	CAATGGCGT	GGATAGCGGT	TTGACTCACG
9250	9260	9270	9280	9290	9300
GGGATTTCCA	AGTCTCCACC	CCATTGACGT	CAATGGGAGT	TTGTTTGGC	ACCAAAATCA
9310	9320	9330	9340	9350	9360
ACGGGACTTT	CCAAAATGTC	GTAACAACTC	CGCCCCATTG	ACGCAAATGG	GCGTAGGCG
9370	9380	9390	9400	9410	9420
TGTACGGTGG	GAGGTCTATA	TAAGCAGAGC	TGGGTACGTG	AACCGTCAGA	TCGCCTGGAG
9430	9440	9450	9460	9470	9480
ACGCCGTCGA	CATGGGTTGG	AGCCTCATCT	TGCTCTTCCT	TGTGCTGTG	GCTACGCGTG
9490	9500	9510	9520	9530	9540
. CCTGTCCGA	GGTGCAGCTG	GTGGAGCTG	GGGGCGGCTT	GGCAAAGCCT	GGGGGGTCCC
9550	9560	9570	9580	9590	9600
TGAGACTCTC	CTGCGCAGCC	TCCGGGTTCA	GGTCACCTT	CAATAACTAC	TACATGGACT
9610	9620	9630	9640	9650	9660
GGGTCCGCCA	GGCTCCAGGG	CAGGGGCTGG	AGTGGGTCTC	ACGTATTAGT	AGTAGTGGTG
9670	9680	9690	9700	9710	9720
ATCCCACATG	GTACGCAGAC	TCCGTGAAGG	GCAGATTAC	CATCTCCAGA	GAGAACGCCA
9730	9740	9750	9760	9770	9780
AGAACACACT	GTTTCTCAA	ATGAACAGCC	TGAGAGCTGA	GGACACGGCT	GTCTATTACT
9790	9800	9810	9820	9830	9840
GTGCGAGCTT	GAECTACAGGG	TCTGACTCCT	GGGGCCAGGG	AGTCCTGGTC	ACCGTCTCCT
9850	9860	9870	9880	9890	9900
LAGCTAGCAC	CAAGGGCCA	TCGGTCTTCC	CCCTGGCACC	CTCCCTCCAAG	AGCACCTCTG
9910	9920	9930	9940	9950	9960
GGGGCACAGC	GGCCCTGGGC	TGCCTGGTCA	AGGACTACTT	CCCCGAACCG	GTGACGGTGT
9970	9980	9990	10000	10010	10020
CGTGGAACTC	AGGCGCCCTG	ACCAGCAGCG	TGACACACCTT	CCCGCTGTG	CTACAGTCCT
10030	10040	10050	10060	10070	10080
CAGGACTCTA	CTCCCTCAGC	AGCGTGGTGA	CCGTGCCCTC	CAGCAGCTTG	GGCACCCAGA
10090	10100	10110	10120	10130	10140
CCTACATCTG	CAACGTGAAT	CACAAGCCA	GCAACACAA	GGTGGACAAG	AAAGTTGAGC
10150	10160	10170	10180	10190	10200
CCAAATCTG	TGACAAAACT	CACACATGCC	CACCGTGCCTC	AGCACCTGAA	CTCCTGGGGG
10210	10220	10230	10240	10250	10260
GACCGTCAGT	CTTCCTCTTC	CCCCAAAAC	CCAAGGACAC	CCTCATGATC	TCCCGGACCC
10270	10280	10290	10300	10310	10320
CTGAGGTAC	ATGCGTGGTG	GTGGACGTGA	GCCACGAAGA	CCCTGAGGTC	AAGTTCAACT
10330	10340	10350	10360	10370	10380
GGTACGTGGA	CGGCGTGGAG	GTGCATAATG	CCAAGACAA	GCCGCGGGAG	GAGCAGTACA
10390	10400	10410	10420	10430	10440

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ACAGCACGTA CGGTGTGGTC AGCGTCTCA CGTCCTGCA CCAGGACTGG CTGAATGGCA

10450 10460 10470 10480 10490 10500
AGGAGTACAA GTGCAAGGTC TCCAACAAAG CCCTCCCAGC CCCCATCGAG AAAACCATCT

10510 10520 10530 10540 10550 10560
CCAAAGCCAA AGGGCAGCCC CGAGAACAC AGGTGTACAC CCTGCCCCA TCCCGGGATG

10570 10580 10590 10600 10610 10620
AGCTGACCAA GAACCAGGTC AGCCTGACCT GCCTGGTCAA AGGCTTCTAT CCCAGCGACA

10630 10640 10650 10660 10670 10680
TCGCCGTGGA GTGGGAGAGC AATGGGCAGC CGGAGAACAA CTACAAGACC ACGGCTCCCG

10690 10700 10710 10720 10730 10740
TGCTGGACTC CGACGGCTCC TTCTTCCTCT ACAGCAAGCT CACCGTGGAC AAGAGCAGGT

10750 10760 10770 10780 10790 10800
GCAGCAGGG GAACGTCTTC TCATGCTCCG TGATGCATGA GGCTCTGCAC AACCACTACA

10810 10820 10830 10840 10850 10860
CGCAGAAGAG CCTCTCCCTG TCTCCGGTA AATGAGGATC CGTTAACGGT TACCAACTAC

10870 10880 10890 10900 10910 10920
CTAGACTGGA TTCGTGACAA CATGCGGGCG TGATATCTAC GTATGATCAG CCTCGACTGT

10930 10940 10950 10960 10970 10980
GCCTTCTAGT TGCCAGCCAT CTGTTGTTTG CCCCTCCCCC GTGCCCTCCT TGACCCCTGGA

10990 11000 11010 11020 11030 11040
AGGTGCCACT CCCACTGTCC TTTCTTAATA AAATGAGGAA ATTGCATCGC ATTGTCTGAG

11050 11060 11070 11080 11090 11100
TAGGTGTCA TCTATTCTGG GGGGTGGGGT GGGGCAGGAC AGCAAGGGGG AGGATTGGGA

11110 11120 11130 11140 11150 11160
ACAATAGC AGGCATGCTG GGGATGCCGT GGGCTCTATG GCTTCTGAGG CGGAAAGAAC

11170 11180 11190 11200 11210 11220
CAGCTGGGGC TCGACAGCAA CGCTAGGTG AGGCCGCTAC TAACTCTCTC CTCCCTCCTT

11230 11240 11250 11260 11270 11280
TTTCTGCA GACGAGGCAG CGCGGCTATC GTGGCTGGCC ACGACGGGCG TTCCCTGCGC

11290 11300 11310 11320 11330 11340
AGCTGTGCTC GACGTGTCA CTGAAGCGGG AAGGGACTGG CTGCTATTGG GCGAAGTGCC

11350 11360 11370 11380 11390 11400
GGGGCAGGAT CTCCCTGTCAT CTCACCTTGC TCCTGCCAG AAAGTATCCA TCATGGCTGA

11410 11420 11430 11440 11450 11460
TGCAATGCGG CGGCTGCATA CGCTTGATCC GGCTACCTGC CCATTCGACC ACCAAGCGAA

11470 11480 11490 11500 11510 11520
ACATCGCATC GAGCGAGCAC GTACTCGGAT GGAAGCCGGT CTTGTGATC AGGATGATCT

11530 11540 11550 11560 11570 11580
GGACGAAGAG CATCAGGGGC TCGCGCCAGC CGAACTGTTC GCCAGGTAAG TGAGCTCCAA

11590 11600 11610 11620 11630 11640
TTCAAGCTCT CGAGCTAGGG CGGCCAGCTA GTAGCTTGC TTCTCAATT CTTATTGCA

11650 11660 11670 11680 11690 11700
TAATGAGAAA AAAAGGAAAA TTAATTTAA CACCAATTCA GTAGTTGATT GAGCAAATGC

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11710	11720	11730	11740	11750	11760
GTTGCCAAA AGGATGCTT AGAGACAGTG TTCTCTGCAC AGATAAGGAC AAACATTATT					
11770	11780	11790	11800	11810	11820
CAGAGGGAGT ACCCAGAGCT GAGACTCCTA AGCCAGTGAG TGGCACAGCA TCCAGGGAGA					
11830	11840	11850	11860	11870	11880
AATATGCTTG TCATCACCGA AGCCTGATTC CGTAGAGCCA CACCCCTGGTA AGGGCCAATC					
11890	11900	11910	11920	11930	11940
TGCTCACACA GGATAGAGAG GGCAGGAGCC AGGGCAGAGC ATATAAGGTG AGGTAGGATC					
11950	11960	11970	11980	11990	12000
AGTTGCTCCT CACATTTGCT TCTGACATAG TTGTGTTGGG AGCTTGGATA GCTTGGGGGG					
12010	12020	12030	12040	12050	12060
GGGCACAGCTC AGGGCTGCGA TTTCGGGCCA AACTTGACGG CAATCCTAGC GTGAAGGCTG					
12070	12080	12090	12100	12110	12120
AGGATTTT ATCCCCGCTG CCATCATGGT TCGACCATTG AACTGCATCG TCGCCGTGTC					
12130	12140	12150	12160	12170	12180
.CCAAAATATG GGGATTGGCA AGAACGGAGA CCTACCCCTGG CCTCCGCTCA GGAACGAGTT					
12190	12200	12210	12220	12230	12240
CAAGTACTTC CAAAGAATGA CCACAACCTC TTCAGTGGAA GGTAAACAGA ATCTGGTGAT					
12250	12260	12270	12280	12290	12300
TATGGGTAGG AAAACCTGGT TCTCCATTCC TGAGAAGAAT CGACCTTAA AGGACAGAAT					
12310	12320	12330	12340	12350	12360
TAATATAGTT CTCAGTAGAG AACTCAAAGA ACCACCAACGA GGAGCTCATT TTCTGCCAA					
12370	12380	12390	12400	12410	12420
AAGTTTGAT GATGCCCTAA CGTAGGGCGCG CCATTAAGAC TTATTGAACA ACCGGAATTG					
12430	12440	12450	12460	12470	12480
.CAAGTAAAG TAGACATGGT TTGGATAGTC GGAGGCAGTT CTGTTTACCA GGAAGCCATG					
12490	12500	12510	12520	12530	12540
AATCAACCAAG GCCACCTCAG ACTCTTTGTG ACAAGGATCA TGCAAGGAAATT TGAAAGTGAC					
12550	12560	12570	12580	12590	12600
ACGTTTTTCC CAGAAATTGA TTTGGGGAAA TATAAACTTC TCCCAGAATA CCCAGGCCTC					
12610	12620	12630	12640	12650	12660
CTCTCTGAGG TCCAGGAGGA AAAAGGCATC AAGTATAAGT TTGAAGTCTA CGAGAAGAAA					
12670	12680	12690	12700	12710	12720
GACTAACAGG AAGATGCTTT CAAGTCTCT GCTCCCCCTCC TAAAGCTATG CATTTTTATA					
12730	12740	12750	12760	12770	12780
AGACCATGGG ACTTTGCTG GCTTAGATC AGCTCGACT GTGCCCTCTA GTGCCAGCC					
12790	12800	12810	12820	12830	12840
ATCTGTTGTT TGCCCTCCC CCGTGCTTC CTTGACCTTG GAAGGTGCCA CTCCCACTGT					
12850	12860	12870	12880	12890	12900
CCTTTCTAA TAAAATGAGG AAATTGCATC GCATTGTCTG AGTAGGTGTC ATTCTATTCT					
12910	12920	12930	12940	12950	12960
GGGGGGTGGG GTGGGGCAGG ACAGCAAGGG GGAGGATTGG GAAGACAATA GCAGGCATGC					
12970	12980	12990	13000	13010	13020
TGGGGATGCG GTGGGCTCTA TGGCTTCTGA GGCGGAAAGA ACCAGCTGGG GCTCGAAGCG					

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13030	13040	13050	13060	13070	13080
GCCGCCATT	TCGCTGGTGG	TCAGATGCGG	GATGGCGTGG	GACGCCGG	GGAGCGTCAC
13090	13100	13110	13120	13130	13140
ACTGAGGTTT	TCCGCCAGAC	GCCACTGCTG	CCAGGGCCTG	ATGTGCCCGG	CTTCTGACCA
13150	13160	13170	13180	13190	13200
TGCGGTCGCG	TTCGGTTGCA	CTACCGTAC	TGTGAGCCAG	AGTTGCCCGG	CGCTCTCCGG
13210	13220	13230	13240	13250	13260
CTGCGTAGT	TCAGGCAGTT	CAATCAACTG	TTTACCTTGT	GGAGCGACAT	CCAGAGGCAC
13270	13280	13290	13300	13310	13320
TTCACCGCTT	GCCAGCGGCT	TACCATCCAG	CGCCACCATC	CAGTGCAGGA	GCTCGTTATC
13330	13340	13350	13360	13370	13380
GCTATGACGG	AACAGGTATT	CGCTGGTCAC	TTCGATGGTT	TGCCCCGATA	AACGGAAC TG
13390	13400	13410	13420	13430	13440
AAAACATGC	TGCTGGTGTT	TTGCTTCCGT	CAGCGCTGGA	TGCGGCGTGC	GGTCGGCAA
13450	13460	13470	13480	13490	13500
GACCAGACCG	TTCATACAGA	ACTGGCGATC	GTTCGGCGTA	TCGCCAAAT	CACCGCCGTA
13510	13520	13530	13540	13550	13560
AGCCGACAC	GGGTTGCCGT	TTTCATCATA	TTTAATCAGC	GACTGATCCA	CCCAGTCCC
13570	13580	13590	13600	13610	13620
GACGAAGCCG	CCCTGTAAAC	GGGGATACTG	ACGAAACGCC	TGCCAGTATT	TAGCGAAACC
13630	13640	13650	13660	13670	13680
GCCAAGACTG	TTACCCATCG	CGTGGGGCGTA	TTCGCAAAGG	ATCAGCGGGC	GCGTCTCTCC
13690	13700	13710	13720	13730	13740
AGGTAGCGAA	AGCCATTTTT	TGATGGACCA	TTTCGGCACA	GCCGGGAAGG	GCTGGTCTTC
13750	13760	13770	13780	13790	13800
~GCCACCGCG	GCGTACATCG	GGCAAATAAT	ATCGGTGGCC	GTGGTGTGCG	CTCCGCCGCC
13810	13820	13830	13840	13850	13860
TTCATACTGC	ACCGGGCGGG	AAGGATCGAC	AGATTTGATC	CAGCGATAAC	GCGCGTCGTG
13870	13880	13890	13900	13910	13920
ATTAGCGCCG	TGGCCTGATT	CATTCCCCAG	CGACCAGATG	ATCACACTCG	GGTGATTACG
13930	13940	13950	13960	13970	13980
ATCGCGCTGC	ACCATTGCG	TTACCGTTC	GCTCATCGCC	GGTAGCCAGC	GCGGATCATC
13990	14000	14010	14020	14030	14040
GGTCAGACGA	TTCATTGGCA	CCATGCCGTG	GGTTTCAATA	TTGGCTTCAT	CCACACATA
14050	14060	14070	14080	14090	14100
CAGGCCGTAG	CGGTGCGACA	GC GTGTACCA	CAGCGGATGG	TTCGGATAAT	GCGAACAGCG
14110	14120	14130	14140	14150	14160
CACGGCGTTA	AA GTTGTCT	GCTTCATCG	CAGGATATCC	TGCACCATCG	TCTGCTCATC
14170	14180	14190	14200	14210	14220
CATGACCTGA	CCATGCGAG	GATGATGCTC	GTGACGGTTA	ACGCCCTGAA	TCAGCAACGG
14230	14240	14250	14260	14270	14280
CTTGCCGTT	AGCAGCAGCA	GACCATTTC	AATCCGCACC	TCGCGGAAAC	CGACATCGCA
14290	14300	14310	14320	14330	14340

DNASIS
Mandy E8N-SHL

GGCTTCTGCT TCAATCAGCG TGCCGTGGC GGTGTGCAGT TCAACCACCG CACGATAGAG

14350	14360	14370	14380	14390	14400
ATTGGGATT	TCGGCGCTCC	ACAGTTTCCG	GTTTCGACG	TTCAGACGTA	GTGTGACGCG

14410	14420	14430	14440	14450	14460
ATCGGCATAA	CCACCAACGCT	CATCGATAAT	TTCACCGCCG	AAAGGCGCGG	TGCCGCTGGC

14470	14480	14490	14500	14510	14520
GACCTGCGTT	TCACCCCTGCC	ATAAAGAAC	TGTTACCGT	AGGTAGTCAC	GCAACTCGCC

14530	14540	14550	14560	14570	14580
GCACATCTGA	ACTTCAGCCT	CCAGTACAGC	GCGGCTGAAA	TCATCATTAA	AGCGAGTGGC

14590	14600	14610	14620	14630	14640
AACATGGAAA	TCGCTGATTT	GTGAGTCGG	TTTATGCAGC	AACGAGACGT	CACGGAAAAT

14650	14660	14670	14680	14690	14700
~CGCTCATC	CGCCACATAT	CCTGATCTTC	CAGATAACTG	CCGTCACTCC	AGCGCAGCAC

14710	14720	14730	14740	14750	14760
CATCACCGCG	AGGC GGTTTT	CTCCGGCGCG	TAAAAATGCG	CTCAGGTCAA	ATTCAGACGG

14770	14780	14790	14800	14810	14820
CAAACGACTG	TCCTGGCCGT	AACCGACCCA	GCGCCCGTTG	CACCAACAGAT	GAAACGCCGA

14830	14840	14850	14860	14870	14880
GTAAACGCCA	TCAAAAATAA	TTCGCGTCTG	GCCTTCCTGT	AGCCAGCTT	CATCAACATT

14890	14900	14910	14920	14930	14940
AAATGTGAGC	GAGTAACAAAC	CCGTCGGATT	CTCCGTGGGA	ACAAACGGCG	GATTGACCGT

14950	14960	14970	14980	14990	15000
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15010	15020	15030	15040	15050	15060
~GGACGACG	ACAGTATCGG	CCTCAGGAAG	ATCGCACTCC	AGCCAGCTT	CCGGCACCGC

15070	15080	15090	15100	15110	15120
TTCTGGTGCC	GGAAACCAGG	CAAAGCGCCA	TTGCCATTTC	AGGCTGCAGCA	ACTGTTGGGA

15130	15140	15150	15160	15170	15180
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15190	15200	15210	15220	15230	15240
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15250	15260	15270	15280	15290	15300
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15310	15320	15330	15340	15350	15360
GCCGGTGCCC	ACAATCGTGC	GCGAACAAAC	TAAACCAGAA	CAAATTATAC	CGGGCGCAC

15370	15380	15390	15400	15410	15420
GCCGCCACCA	CCTTCTCCCG	TGCCTAACAT	TCCAGCGCCT	CCACCACAC	CACCAACATC

15430	15440	15450	15460	15470	15480
GATGTCTGAA	TTGCCGCCCG	CTCCACCAAT	GCCGACGGAA	CCTCAACCCG	CTGCACCTTT

15490	15500	15510	15520	15530	15540
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15550	15560	15570	15580	15590	15600
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DNASIS
Mandy E8N-SHL

15610 15620 15630 15640 15650 15660
 GCCTAAAGAG ACATTTGAGC CTAAACGCC GTCTGCATCA CCGCCACAC CTCCGCCTCC

 15670 15680 15690 15700 15710 15720
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 15730 15740 15750 15760 15770 15780
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 15850 15860 15870 15880 15890 15900
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 15910 15920 15930 15940 15950 15960
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 15970 15980 15990 16000 16010 16020
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 16030 16040 16050 16060 16070 16080
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 16090 16100 16110 16120 16130 16140
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 16150 16160 16170 16180 16190 16200
 GCTCCGTGCG TTGAGGACCC GGCTAGGCTG GCGGGGTTGC CTTACTGGTT AGCAGAACGA

 16210 16220 16230 16240 16250 16260
 ATCACCGATA CGCGAGCGA CGTGAAGCGA CTGCTGCTGC AAAACGTCG CGACCTGAGC

 16270 16280 16290 16300 16310 16320
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 16330 16340 16350 16360 16370 16380
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 16390 16400 16410 16420 16430 16440
 ACCTACATCT GTATTAACGA AGCGCTGGCA TTGACCCCTGA GTGATTTTC TCTGGTCCCG

 16450 16460 16470 16480 16490 16500
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 16510 16520 16530 16540 16550 16560
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 16570 16580 16590 16600 16610 16620
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 16630 16640 16650 16660 16670 16680
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 16690 16700 16710 16720 16730 16740
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 16750 16760 16770 16780 16790 16800
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 16810 16820 16830 16840 16850 16860
 GGTACACAGCT TGTCTGTAAG CGGATGCCGG GAGCAGACAA GCCCGTCAGG GCGCGTCAGC

 16870 16880 16890 16900 16910 16920
 GGGTGTGGC GGGTGTGGG GCGCAGCCAT GACCCAGTCA CGTAGCGATA GCGGAGTGTAA

DNASIS
Mandy SE8N-SHL

16930	16940	16950	16960	16970	16980
TACTGGCTTA ACTATGCGGC ATCAGAGCAG ATTGTACTGA GAGTGCACCA TATGCGGTGT					
16990	17000	17010	17020	17030	17040
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17050	17060	17070	17080	17090	17100
CTCACTGACT CGCTGCGCTC GGTCGTTCCG CTGGGGCGAG CGGTATCAGC TCACTCAAAG					
17110	17120	17130	17140	17150	17160
GCGGTAATAAC GGTTATCCAC AGAATCAGGG GATAACGCAG GAAAGAACAT GTGAGCAAAA					
17170	17180	17190	17200	17210	17220
GGCCAGCAAA AGGCCAGGAA CCGTAAAAAG GCCCGCGTTGC TGGCGTTTTT CCATAGGCTC					
17230	17240	17250	17260	17270	17280
CGCCCCCTG ACGAGCATCA CAAAAATCGA CGCTCAAGTC AGAGGTGGCG AAACCCGACA					
17290	17300	17310	17320	17330	17340
GGACTATAAA GATACCAGGC GTTTCCCCCT GGAAGCTCCC TCGTGCCTC TCCTGTTCCG					
17350	17360	17370	17380	17390	17400
ACCTGCGCGC TTACCGGATA CCTGTCCGCC TTTCTCCCTT CGGGAAAGCGT GGCGCTTTCT					
17410	17420	17430	17440	17450	17460
CATAGCTCAC GCTGTAGGTA TCTCAGTTCG GTGTAGGTCG TTCGCTCCAA GCTGGGCTGT					
17470	17480	17490	17500	17510	17520
GTGACACGAAC CCCCCGTTCA GCCCGACCGC TGCGCCTTAT CGGTAACTA TCGTCTTGAG					
17530	17540	17550	17560	17570	17580
TCCAACCCGG TAAGACACGA CTTATGCCA CTGGCAGCAG CCACTGGTAA CAGGATTAGC					
17590	17600	17610	17620	17630	17640
AGAGCGAGGT ATGTAGGCAG TGCTACAGAG TTCTTGAAGT GGTGGCTAA CTACGGCTAC					
17650	17660	17670	17680	17690	17700
ACTAGAAGGA CAGTATTTGG TATCTGCCT CTGCTGAAGC CAGTTACCTT CGGAAAAAGA					
17710	17720	17730	17740	17750	17760
GTTGGTAGCT CTTGATCCGG CAAACAAACC ACCGCTGGTA GCGGTGGTTT TTTTGTGTTGC					
17770	17780	17790	17800	17810	17820
AAGCAGCAGA TTACCGCGAG AAAAAAAGGA TCTCAAGAAG ATCCTTTGAT CTTTCTACG					
17830	17840	17850	17860	17870	17880
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17890	17900	17910	17920	17930	17940
AAAAGGATCT TCACCTAGAT CCTTTAAAT TAAAAATGAA GTTTAAATC AATCTAAAGT					
17950	17960	17970	17980	17990	18000
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18010	18020	18030	18040	18050	18060
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18070	18080	18090	18100	18110	18120
ATACGGGAGG GCTTACCATC TGGCCCCAGT GCTGCAATGA TACCGCGAGA CCCACGCTCA					
18130	18140	18150	18160	18170	18180
CCGGCTCCAG ATTTATCAGC AATAAACAG CCAGCCGGAA GGGCCGAGCG CAGAAGTGGT					
18190	18200	18210	18220	18230	18240

DNASIS

Mandy + SE8N-SHL

51 / 51

CCTGCAACTT TATCCGCCTC CATCCAGTCT ATTAATTGTT GCCGGGAAGC TAGAGTAAGT
 18250 18260 18270 18280 18290 18300
 AGTTGCCAG TTAATAGTTT GCGAACGTT GTGCCATTG CTGCAGGCAT CGTGGTGTCA
 18310 18320 18330 18340 18350 18360
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 TGATCCCCA TGTTGTGAA AAAAGCGGTT AGCTCCCTCG GTCCCTCGAT CGTTGTGAGA
 18430 18440 18450 18460 18470 18480
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 18490 18500 18510 18520 18530 18540
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 18550 18560 18570 18580 18590 18600
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 18610 18620 18630 18640 18650 18660
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 18670 18680 18690 18700 18710 18720
 TCAAGGATCT TACCGCTGTT GAGATCCAGT TCGATGTAAC CCACTCGTGC ACCCAACTGA
 18730 18740 18750 18760 18770 18780
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 18790 18800 18810 18820 18830 18840
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 18850 18860 18870 18880 18890 18900
 CAATATTATT GAAGCATTAA TCAGGGTTAT TGTCTCATGA GCGGATACAT ATTTGAATGT
 18910 18920 18930 18940 18950 18960
 ATTTAGAAAA ATAAACAAAT AGGGGTTCCG CGCACATTTC CCCGAAAAGT GCCACCTGAC
 18970 18980 18990 19000 19010 19020
 GTCTAAGAAA CCATTATTAT CATGACATTA ACCTATAAAA ATAGGCCTAT CACGAGGCC
 19030 19040 19050 19060 19070 19080
 TTTCGTCTTC AAGAA.....

INTERNATIONAL SEARCH REPORT

Int. Application No
PCT/US 98/03935

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/90	C12N15/85	C12Q1/68	C12N5/	C12N9/12
C12N15/13	C07K16/28	C12N15/12	C07K14/705	G01N33/53
C12N15/62	C07K19/00			

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C12Q C07K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 94 11523 A (IDEC PHARMACEUTICALS CORPORATION (US); REFF MITCHELL E. (US)) 26 May 1994 cited in the application see abstract see page 9, line 21 - page 10, line 29 see page 41, line 19 - page 42, line 19; figure 6	1,4-8, 11,12, 25-29, 31,32
A	US 5 464 764 A (CAPECCHI MARIO R. AND KIRK THOMAS R.) 7 November 1995 see abstract see column 13, line 32 - column 14, line 5	1
A	WO 94 05784 A (UNITED STATES AMERICA REPRESENTED BY THE SECRETARY US DPT. AGRICULTURE) 17 March 1994 see abstract	1

	-/-	

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Patent family members are listed in annex.

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- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search

23 July 1998

Date of mailing of the international search report

05/08/1998

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Authorized officer

Macchia, G

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 98/03935

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 93 24642 A (TSI CORPORATION (US)) 9 December 1993 see abstract ----	1
A	BARNETT R.S. ET AL.: "Antibody production in chinese hamster ovary cells using an impaired selectable marker" ACS SYMPOSIUM SERIES: ANTIBODY EXPRESSION AND ENGINEERING, vol. 604, 1995, pages 27-40, XP002072464 -----	

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/03935

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9411523	A	26-05-1994	AU 682481 B AU 5613294 A CA 2149326 A DE 669986 T EP 0669986 A ES 2088838 T JP 8503138 T US 5648267 A US 5733779 A		09-10-1997 08-06-1994 26-05-1994 10-10-1996 06-09-1995 01-10-1996 09-04-1996 15-07-1997 31-03-1998
US 5464764	A	07-11-1995	US 5487992 A US 5627059 A US 5631153 A		30-01-1996 06-05-1997 20-05-1997
WO 9405784	A	17-03-1994	AU 4839493 A MX 9305183 A		29-03-1994 31-05-1994
WO 9324642	A	09-12-1993	AU 4401993 A		30-12-1993



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(22) International Filing Date: 9 March 1998 (09.03.98)	
(30) Priority Data:	
08/819,866 14 March 1997 (14.03.97) US	
09/023,715 13 February 1998 (13.02.98) US	
(71) Applicant: IDEC PHARMACEUTICALS CORPORATION [US/US]; 11011 Torreyana Road, San Diego, CA 92121 (US).	
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(74) Agents: GESS, E., Joseph et al.; Burns, Doane, Swecker & Mathis L.L.P., P.O. Box 1404, Alexandria, VA 22313-1404 (US).	

(54) Title: METHOD FOR INTEGRATING GENES AT SPECIFIC SITES IN MAMMALIAN CELLS VIA HOMOLOGOUS RECOMBINATION AND VECTORS FOR ACCOMPLISHING THE SAME

(57) Abstract

A method for achieving site specific integration of a desired DNA at a target site in a mammalian cell via homologous recombination is described. This method provides for the reproducible selection of cell lines wherein a desired DNA is integrated at a predetermined transcriptionally active site previously marked with a marker plasmid. The method is particularly suitable for the production of mammalian cell lines which secrete mammalian proteins at high levels, in particular immunoglobulins. Vectors and vector combinations for use in the subject cloning method are also provided.

Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

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DK	Denmark	LR	Liberia	SG	Singapore		

Title of the Invention

METHOD FOR INTEGRATING GENES AT SPECIFIC SITES IN MAMMALIAN CELLS VIA HOMOLOGOUS RECOMBINATION AND VECTORS FOR ACCOMPLISHING THE SAME

5

Field of the Invention

The present invention relates to a process of targeting the integration of a desired exogenous DNA to a specific location within the genome of a mammalian cell. 10 More specifically, the invention describes a novel method for identifying a transcriptionally active target site ("hot spot") in the mammalian genome, and inserting a desired DNA at this site via homologous recombination. The invention also optionally provides the ability for 15 gene amplification of the desired DNA at this location by co-integrating an amplifiable selectable marker, e.g., DHFR, in combination with the exogenous DNA. The invention additionally describes the construction of novel vectors suitable for accomplishing the above, and 20 further provides mammalian cell lines produced by such methods which contain a desired exogenous DNA integrated at a target hot spot.

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Background

Technology for expressing recombinant proteins in both prokaryotic and eukaryotic organisms is well established. Mammalian cells offer significant advantages over bacteria or yeast for protein production, resulting from their ability to correctly assemble, glycosylate and post-translationally modify recombinantly expressed proteins. After transfection into the host cells, recombinant expression constructs can be maintained as extrachromosomal elements, or may be integrated into the host cell genome. Generation of stably transfected mammalian cell lines usually involves the latter; a DNA construct encoding a gene of interest along with a drug resistance gene (dominant selectable marker) is introduced into the host cell, and subsequent growth in the presence of the drug allows for the selection of cells that have successfully integrated the exogenous DNA. In many instances, the gene of interest is linked to a drug resistant selectable marker which can later be subjected to gene amplification. The gene encoding dihydrofolate reductase (DHFR) is most commonly used for this purpose. Growth of cells in the presence of methotrexate, a competitive inhibitor of DHFR, leads to increased DHFR production by means of amplification of the DHFR gene. As flanking regions of DNA will also become amplified, the resultant coamplification of a DHFR linked gene in the transfected cell line can lead to increased protein

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production, thereby resulting in high level expression of the gene of interest.

While this approach has proven successful, there are a number of problems with the system because of the random nature of the integration event. These problems exist because expression levels are greatly influenced by the effects of the local genetic environment at the gene locus, a phenomena well documented in the literature and generally referred to as "position effects" (for example, see Al-Shawi et al, *Mol. Cell. Biol.*, 10:1192-1198 (1990); Yoshimura et al, *Mol. Cell. Biol.*, 7:1296-1299 (1987)). As the vast majority of mammalian DNA is in a transcriptionally inactive state, random integration methods offer no control over the transcriptional fate of the integrated DNA. Consequently, wide variations in the expression level of integrated genes can occur, depending on the site of integration. For example, integration of exogenous DNA into inactive, or transcriptionally "silent" regions of the genome will result in little or no expression. By contrast integration into a transcriptionally active site may result in high expression.

Therefore, when the goal of the work is to obtain a high level of gene expression, as is typically the desired outcome of genetic engineering methods, it is generally necessary to screen large numbers of transfec-tants to find such a high producing clone.

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Additionally, random integration of exogenous DNA into the genome can in some instances disrupt important cellular genes, resulting in an altered phenotype.

These factors can make the generation of high expressing stable mammalian cell lines a complicated and laborious process.

Recently, our laboratory has described the use of DNA vectors containing translationally impaired dominant selectable markers in mammalian gene expression. (This is disclosed in U.S. Serial No. 08/147,696 filed November 3, 1993, recently allowed).

These vectors contain a translationally impaired neomycin phosphotransferase (neo) gene as the dominant selectable marker, artificially engineered to contain an intron into which a DHFR gene along with a gene or genes of interest is inserted. Use of these vectors as expression constructs has been found to significantly reduce the total number of drug resistant colonies produced, thereby facilitating the screening procedure in relation to conventional mammalian expression vectors.

Furthermore, a significant percentage of the clones obtained using this system are high expressing clones.

These results are apparently attributable to the modifications made to the neo selectable marker. Due to the translational impairment of the neo gene, transfected cells will not produce enough neo protein to survive drug selection, thereby decreasing the overall

- 5 -

number of drug resistant colonies. Additionally, a higher percentage of the surviving clones will contain the expression vector integrated into sites in the genome where basal transcription levels are high,
5 resulting in overproduction of neo, thereby allowing the cells to overcome the impairment of the neo gene. Concomitantly, the genes of interest linked to neo will be subject to similar elevated levels of transcription. This same advantage is also true as a result of the
10 artificial intron created within neo; survival is dependent on the synthesis of a functional neo gene, which is in turn dependent on correct and efficient splicing of the neo introns. Moreover, these criteria are more likely to be met if the vector DNA has
15 integrated into a region which is already highly transcriptionally active.

Following integration of the vector into a transcriptionally active region, gene amplification is performed by selection for the DHFR gene. Using this system,
20 it has been possible to obtain clones selected using low levels of methotrexate (50nM), containing few (<10) copies of the vector which secrete high levels of protein (>55pg/cell/day). Furthermore, this can be achieved in a relatively short period of time. However,
25 the success in amplification is variable. Some transcriptionally active sites cannot be amplified and

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therefore the frequency and extent of amplification from a particular site is not predictable.

Overall, the use of these translationally impaired vectors represents a significant improvement over other methods of random integration. However, as discussed, the problem of lack of control over the integration site remains a significant concern.

One approach to overcome the problems of random integration is by means of gene targeting, whereby the exogenous DNA is directed to a specific locus within the host genome. The exogenous DNA is inserted by means of homologous recombination occurring between sequences of DNA in the expression vector and the corresponding homologous sequence in the genome. However, while this type of recombination occurs at a high frequency naturally in yeast and other fungal organisms, in higher eukaryotic organisms it is an extremely rare event. In mammalian cells, the frequency of homologous versus non-homologous (random integration) recombination is reported to range from 1/100 to 1/5000 (for example, see Capecchi, *Science*, 244:1288-1292 (1989); Morrow and Kucherlapati, *Curr. Op. Biotech.*, 4:577-582 (1993)).

One of the earliest reports describing homologous recombination in mammalian cells comprised an artificial system created in mouse fibroblasts (Thomas et al, *Cell*, 44:419-428 (1986)). A cell line containing a mutated, non-functional version of the neo gene integrated into

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the host genome was created, and subsequently targeted with a second non-functional copy of neo containing a different mutation. Reconstruction of a functional neo gene could occur only by gene targeting. Homologous 5 recombinants were identified by selecting for G418 resistant cells, and confirmed by analysis of genomic DNA isolated from the resistant clones.

Recently, the use of homologous recombination to replace the heavy and light immunoglobulin genes at 10 endogenous loci in antibody secreting cells has been reported. (U.S. Patent No. 5,202,238, Fell et al, (1993).) However, this particular approach is not widely applicable, because it is limited to the production of immunoglobulins in cells which 15 endogenously express immunoglobulins, e.g., B cells and myeloma cells. Also, expression is limited to single copy gene levels because co-amplification after homologous recombination is not included. The method is further complicated by the fact that two separate 20 integration events are required to produce a functional immunoglobulin: one for the light chain gene followed by one for the heavy chain gene.

An additional example of this type of system has 25 been reported in NS/0 cells, where recombinant immunoglobulins are expressed by homologous recombination into the immunoglobulin gamma 2A locus (Hollis et al, international patent application #

PCT/IB95 (00014).) Expression levels obtained from this site were extremely high - on the order of 20pg/cell/day from a single copy integrant. However, as in the above example, expression is limited to this level because an amplifiable gene is not cointegrated in this system.

5 Also, other researchers have reported aberrant glycosylation of recombinant proteins expressed in NS/0 cells (for example, see Flesher et al, *Biotech. and Bioeng.*, 48:399-407 (1995)), thereby limiting the applicability of this approach.

10 The cre-loxP recombination system from bacteriophage P1 has recently been adapted and used as a means of gene targeting in eukaryotic cells.

15 Specifically, the site specific integration of exogenous DNA into the Chinese hamster ovary (CHO) cell genome using cre recombinase and a series of lox containing vectors have been described. (Fukushige and Sauer, *Proc. Natl. Acad. Sci. USA*, 89:7905-7909 (1992).) This system is attractive in that it provides for

20 reproducible expression at the same chromosomal location. However, no effort was made to identify a chromosomal site from which gene expression is optimal, and as in the above example, expression is limited to single copy levels in this system. Also, it is

25 complicated by the fact that one needs to provide for expression of a functional recombinase enzyme in the mammalian cell.

The use of homologous recombination between an introduced DNA sequence and its endogenous chromosomal locus has also been reported to provide a useful means of genetic manipulation in mammalian cells, as well as 5 in yeast cells. (See e.g., Bradley et al, *Meth. Enzymol.*, 223:855-879 (1993); Capecchi, *Science*, 244:1288-1292 (1989); Rothstein et al, *Meth. Enzymol.*, 194:281-301 (1991)). To date, most mammalian gene targeting studies have been directed toward gene 10 disruption ("knockout") or site-specific mutagenesis of selected target gene loci in mouse embryonic stem (ES) cells. The creation of these "knockout" mouse models has enabled scientists to examine specific structure-function issues and examine the biological 15 importance of a myriad of mouse genes. This field of research also has important implications in terms of potential gene therapy applications.

Also, vectors have recently been reported by Cell-tech (Kent, U.K.) which purportedly are targeted to 20 transcriptionally active sites in NSO cells, which do not require gene amplification (Peakman et al, *Hum. Antibod. Hybridomas*, 5:65-74 (1994)). However, levels of immunoglobulin secretion in these unamplified cells have not been reported to exceed 20pg/cell/day, while in 25 amplified CHO cells, levels as high as 100pg/cell/day can be obtained (Id.).

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It would be highly desirable to develop a gene targeting system which reproducibly provided for the integration of exogenous DNA into a predetermined site in the genome known to be transcriptionally active.

5 Also, it would be desirable if such a gene targeting system would further facilitate co-amplification of the inserted DNA after integration. The design of such a system would allow for the reproducible and high level expression of any cloned gene of interest in a mammalian 10 cell, and undoubtedly would be of significant interest to many researchers.

In this application, we provide a novel mammalian expression system, based on homologous recombination occurring between two artificial substrates contained in 15 two different vectors. Specifically, this system uses a combination of two novel mammalian expression vectors, referred to as a "marking" vector and a "targeting" vector.

Essentially, the marking vector enables the identification and marking of a site in the mammalian genome 20 which is transcriptionally active, i.e., a site at which gene expression levels are high. This site can be regarded as a "hot spot" in the genome. After integration of the marking vector, the subject expression system 25 enables another DNA to be integrated at this site, i.e., the targeting vector, by means of homologous recombination occurring between DNA sequences common to

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both vectors. This system affords significant advantages over other homologous recombination systems.

Unlike most other homologous systems employed in mammalian cells, this system exhibits no background.

5 Therefore, cells which have only undergone random integration of the vector do not survive the selection. Thus, any gene of interest cloned into the targeting plasmid is expressed at high levels from the marked hot spot. Accordingly, the subject method of gene expression substantially or completely eliminates the problems inherent to systems of random integration, discussed in detail above. Moreover, this system provides reproducible and high level expression of any recombinant protein at the same transcriptionally active site in the 10 mammalian genome. In addition, gene amplification may be effected at this particular transcriptionally active site by including an amplifiable dominant selectable marker (e.g. DHFR) as part of the marking vector.

Objects of the Invention

20 Thus, it is an object of the invention to provide an improved method for targeting a desired DNA to a specific site in a mammalian cell.

It is a more specific object of the invention to provide a novel method for targeting a desired DNA to a 25 specific site in a mammalian cell via homologous recombination.

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It is another specific object of the invention to provide novel vectors for achieving site specific integration of a desired DNA in a mammalian cell.

5 It is still another object of the invention to provide novel mammalian cell lines which contain a desired DNA integrated at a predetermined site which provides for high expression.

10 It is a more specific object of the invention to provide a novel method for achieving site specific integration of a desired DNA in a Chinese hamster ovary (CHO) cell.

15 It is another more specific object of the invention to provide a novel method for integrating immunoglobulin genes, or any other genes, in mammalian cells at predetermined chromosomal sites that provide for high expression.

20 It is another specific object of the invention to provide novel vectors and vector combinations suitable for integrating immunoglobulin genes into mammalian cells at predetermined sites that provide for high expression.

25 It is another object of the invention to provide mammalian cell lines which contain immunoglobulin genes integrated at predetermined sites that provide for high expression.

It is an even more specific object of the invention to provide a novel method for integrating immunoglobulin

genes into CHO cells that provide for high expression, as well as novel vectors and vector combinations that provide for such integration of immunoglobulin genes into CHO cells.

5 In addition, it is a specific object of the invention to provide novel CHO cell lines which contain immunoglobulin genes integrated at predetermined sites that provide for high expression, and have been amplified by methotrexate selection to secrete even greater amounts
10 of functional immunoglobulins.

Brief Description of the Figures

Figure 1 depicts a map of a marking plasmid according to the invention referred to as Desmond. The plasmid is shown in circular form (1a) as well as a
15 linearized version used for transfection (1b).

Figure 2(a) shows a map of a targeting plasmid referred to "Molly". Molly is shown here encoding the anti-CD20 immunoglobulin genes, expression of which is described in Example 1.

20 Figure 2(b) shows a linearized version of Molly, after digestion with the restriction enzymes *Kpn*1 and *Pac*1. This linearized form was used for transfection.

Figure 3 depicts the potential alignment between Desmond sequences integrated into the CHO genome, and
25 incoming targeting Molly sequences. One potential ar-

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rangement of Molly integrated into Desmond after homologous recombination is also presented.

Figure 4 shows a Southern analysis of single copy Desmond clones. Samples are as follows:

- 5 Lane 1: λ HindIII DNA size marker
- Lane 2: Desmond clone 10F3
- Lane 3: Desmond clone 10C12
- Lane 4: Desmond clone 15C9
- Lane 5: Desmond clone 14B5
- 10 Lane 6: Desmond clone 9B2

Figure 5 shows a Northern analysis of single copy Desmond clones. Samples are as follows: Panel A: northern probed with CAD and DHFR probes, as indicated on the figure. Panel B: duplicate northern, probed with CAD and HisD probes, as indicated. The RNA samples loaded in panels A and B are as follows:

- Lane 1: clone 9B2, lane 2; clone 10C12, lane 3; clone 14B5, lane 4; clone 15C9, lane 5; control RNA from CHO transfected with a HisD and DHFR containing plasmid,
- 20 lane 6; untransfected CHO.

Figure 6 shows a Southern analysis of clones resulting from the homologous integration of Molly into Desmond. Samples are as follows:

- Lane 1: λ HindIII DNA size markers, Lane 2: 20F4, lane 3; 25
- 25 5F9, lane 4; 21C7, lane 5; 24G2, lane 6; 25E1, lane 7; 28C9, lane 8; 29F9, lane 9; 39G11, lane 10; 42F9, lane 11; 50G10, lane 12; Molly plasmid DNA, linearized with

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BglII (top band) and cut with BglII and KpnI (lower band), lane 13; untransfected Desmond.

Figures 7A through 7G contain the Sequence Listing for Desmond.

5 Figures 8A through 8I contain the Sequence Listing for Molly-containing anti-CD20.

Figure 9 contains a map of the targeting plasmid, "Mandy," shown here encoding anti-CD23 genes, the expression of which is disclosed in Example 5.

10 Figures 10A through 10N contain the sequence listing of "Mandy" containing the anti-CD23 genes as disclosed in Example 5.

Detailed Description of the Invention

The invention provides a novel method for integrating 15 a desired exogenous DNA at a target site within the genome of a mammalian cell via homologous recombination. Also, the invention provides novel vectors for achieving the site specific integration of a DNA at a target site in the genome of a mammalian cell.

20 More specifically, the subject cloning method provides for site specific integration of a desired DNA in a mammalian cell by transfection of such cell with a "marker plasmid" which contains a unique sequence that is foreign to the mammalian cell genome and which 25 provides a substrate for homologous recombination, followed by transfection with a "target plasmid" containing

a sequence which provides for homologous recombination with the unique sequence contained in the marker plasmid, and further comprising a desired DNA that is to be integrated into the mammalian cell. Typically, the 5 integrated DNA will encode a protein of interest, such as an immunoglobulin or other secreted mammalian glycoprotein.

The exemplified homologous recombination system uses the neomycin phosphotransferase gene as a dominant 10 selectable marker. This particular marker was utilized based on the following previously published observations;

(i) the demonstrated ability to target and restore function to a mutated version of the neo gene (cited 15 earlier) and

(ii) our development of translationally impaired expression vectors, in which the neo gene has been artificially created as two exons with a gene of interest inserted in the intervening intron; neo exons are correctly spliced and translated in vivo, producing a functional protein and thereby conferring G418 resistance on the resultant cell population. In this application, the neo gene is split into three exons. The third exon of 20 neo is present on the "marker" plasmid and becomes integrated into the host cell genome upon integration of the 25 marker plasmid into the mammalian cells. Exons 1 and 2 are present on the targeting plasmid, and are separated

by an intervening intron into which at least one gene of interest is cloned. Homologous recombination of the targeting vector with the integrated marking vector results in correct splicing of all three exons of the 5 neo gene and thereby expression of a functional neo protein (as determined by selection for G418 resistant colonies). Prior to designing the current expression system, we had experimentally tested the functionality of such a triply spliced neo construct in mammalian 10 cells. The results of this control experiment indicated that all three neo exons were properly spliced and therefore suggested the feasibility of the subject invention.

However, while the present invention is exemplified 15 using the neo gene, and more specifically a triple split neo gene, the general methodology should be efficacious with other dominant selectable markers.

As discussed in greater detail *infra*, the present invention affords numerous advantages to conventional 20 gene expression methods, including both random integration and gene targeting methods. Specifically, the subject invention provides a method which reproducibly allows for site-specific integration of a desired DNA into a transcriptionally active domain of a mammalian 25 cell. Moreover, because the subject method introduces an artificial region of "homology" which acts as a unique substrate for homologous recombination and the

insertion of a desired DNA, the efficacy of subject invention does not require that the cell endogenously contain or express a specific DNA. Thus, the method is generically applicable to all mammalian cells, and can
5 be used to express any type of recombinant protein.

The use of a triply spliced selectable marker, e.g., the exemplified triply spliced neo construct, guarantees that all G418 resistant colonies produced will arise from a homologous recombination event (random integrants will not produce a functional neo gene and consequently will not survive G418 selection). Thus,
10 the subject invention makes it easy to screen for the desired homologous event. Furthermore, the frequency of additional random integrations in a cell that has undergone a homologous recombination event appears to be low.
15

Based on the foregoing, it is apparent that a significant advantage of the invention is that it substantially reduces the number of colonies that need be screened to identify high producer clones, i.e., cell
20 lines containing a desired DNA which secrete the corresponding protein at high levels. On average, clones containing integrated desired DNA may be identified by screening about 5 to 20 colonies (compared to several thousand which must be screened when using standard
25 random integration techniques, or several hundred using the previously described intronic insertion vectors) Additionally, as the site of integration was preselected

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and comprises a transcriptionally active domain, all exogenous DNA expressed at this site should produce comparable, i.e. high levels of the protein of interest.

Moreover, the subject invention is further advantageous in that it enables an amplifiable gene to be inserted on integration of the marking vector. Thus, when a desired gene is targeted to this site via homologous recombination, the subject invention allows for expression of the gene to be further enhanced by gene amplification. In this regard, it has been reported in from the literature that different genomic sites have different capacities for gene amplification (Meinkoth et al, *Mol. Cell Biol.*, 7:1415-1424 (1987)). Therefore, this technique is further advantageous as it allows for the placement of a desired gene of interest at a specific site that is both transcriptionally active and easily amplified. Therefore, this should significantly reduce the amount of time required to isolate such high producers.

Specifically, while conventional methods for the construction of high expressing mammalian cell lines can take 6 to 9 months, the present invention allows for such clones to be isolated on average after only about 3-6 months. This is due to the fact that conventionally isolated clones typically must be subjected to at least three rounds of drug resistant gene amplification in order to reach satisfactory levels of gene expression.

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As the homologously produced clones are generated from a preselected site which is a high expression site, fewer rounds of amplification should be required before reaching a satisfactory level of production.

5 Still further, the subject invention enables the reproducible selection of high producer clones wherein the vector is integrated at low copy number, typically single copy. This is advantageous as it enhances the stability of the clones and avoids other potential adverse side-effects associated with high copy number. As described *supra*, the subject homologous recombination system uses the combination of a "marker plasmid" and a "targeting plasmid" which are described in more detail below.

15 The "marker plasmid" which is used to mark and identify a transcriptionally hot spot will comprise at least the following sequences:

(i) a region of DNA that is heterologous or unique to the genome of the mammalian cell, which functions as a source of homology, allows for homologous recombination (with a DNA contained in a second target plasmid). More specifically, the unique region of DNA (i) will generally comprise a bacterial, viral, yeast synthetic, or other DNA which is not normally present in the 25 mammalian cell genome and which further does not comprise significant homology or sequence identity to DNA contained in the genome of the mammalian cell.

Essentially, this sequence should be sufficiently different to mammalian DNA that it will not significantly recombine with the host cell genome via homologous recombination. The size of such unique DNA 5 will generally be at least about 2 to 10 kilobases in size, or higher, more preferably at least about 10kb, as several other investigators have noted an increased frequency of targeted recombination as the size of the homology region is increased (Capecchi, *Science*, 10 244:1288-1292 (1989)).

The upper size limit of the unique DNA which acts as a site for homologous recombination with a sequence in the second target vector is largely dictated by potential stability constraints (if DNA is too large it 15 may not be easily integrated into a chromosome and the difficulties in working with very large DNAs.

(ii) a DNA including a fragment of a selectable marker DNA, typically an exon of a dominant selectable marker gene. The only essential feature of this DNA is 20 that it not encode a functional selectable marker protein unless it is expressed in association with a sequence contained in the target plasmid. Typically, the target plasmid will comprise the remaining exons of the dominant selectable marker gene (those not comprised in 25 "targeting" plasmid). Essentially, a functional selectable marker should only be produced if homologous recombination occurs (resulting in the association and

expression of this marker DNA (i) sequence together with the portion(s) of the selectable marker DNA fragment which is (are) contained in the target plasmid).

As noted, the current invention exemplifies the
5 use of the neomycin phosphotransferase gene as the dominant selectable marker which is "split" in the two vectors. However, other selectable markers should also be suitable, e.g., the *Salmonella histidinol dehydrogenase* gene, *hygromycin phosphotransferase* gene, *herpes simplex* 10 virus thymidine kinase gene, adenosine deaminase gene, glutamine synthetase gene and hypoxanthine-guanine phosphoribosyl transferase gene.

(iii) a DNA which encodes a functional selectable marker protein, which selectable marker is different
15 from the selectable marker DNA (ii). This selectable marker provides for the successful selection of mammalian cells wherein the marker plasmid is successfully integrated into the cellular DNA. More preferably, it is desirable that the marker plasmid comprise two such
20 dominant selectable marker DNAs, situated at opposite ends of the vector. This is advantageous as it enables integrants to be selected using different selection agents and further enables cells which contain the entire vector to be selected. Additionally, one marker
25 can be an amplifiable marker to facilitate gene amplification as discussed previously. Any of the

dominant selectable marker listed in (ii) can be used as well as others generally known in the art.

Moreover, the marker plasmid may optionally further comprise a rare endonuclease restriction site. This is potentially desirable as this may facilitate cleavage. If present, such rare restriction site should be situated close to the middle of the unique region that acts as a substrate for homologous recombination. Preferably such sequence will be at least about 12 nucleotides.

The introduction of a double stranded break by similar methodology has been reported to enhance the frequency of homologous recombination. (Choulika et al, *Mol. Cell. Biol.*, 15:1968-1973 (1995)). However, the presence of such sequence is not essential.

The "targeting plasmid" will comprise at least the following sequences:

(1) the same unique region of DNA contained in the marker plasmid or one having sufficient homology or sequence identity therewith that said DNA is capable of combining via homologous recombination with the unique region (i) in the marker plasmid. Suitable types of DNAs are described *supra* in the description of the unique region of DNA (1) in the marker plasmid.

(2) The remaining exons of the dominant selectable marker, one exon of which is included as (ii) in the marker plasmid listed above. The essential features of this DNA fragment is that it result in a functional

(selectable) marker protein only if the target plasmid integrates via homologous recombination (wherein such recombination results in the association of this DNA with the other fragment of the selectable marker DNA contained in the marker plasmid) and further that it allow for insertion of a desired exogenous DNA. Typically, this DNA will comprise the remaining exons of the selectable marker DNA which are separated by an intron. For example, this DNA may comprise the first two exons of the neo gene and the marker plasmid may comprise the third exon (back third of neo).

(3) The target plasmid will also comprise a desired DNA, e.g., one encoding a desired polypeptide, preferably inserted within the selectable marker DNA fragment contained in the plasmid. Typically, the DNA will be inserted in an intron which is comprised between the exons of the selectable marker DNA. This ensures that the desired DNA is also integrated if homologous recombination of the target plasmid and the marker plasmid occurs. This intron may be naturally occurring or it may be engineered into the dominant selectable marker DNA fragment.

This DNA will encode any desired protein, preferably one having pharmaceutical or other desirable properties. Most typically the DNA will encode a mammalian protein, and in the current examples provided, an immunoglobulin or an immunoadhesin. However the

invention is not in any way limited to the production of immunoglobulins.

As discussed previously, the subject cloning method is suitable for any mammalian cell as it does not require for efficacy that any specific mammalian sequence or sequences be present. In general, such mammalian cells will comprise those typically used for protein expression, e.g., CHO cells, myeloma cells, COS cells, BHK cells, Sp2/0 cells, NIH 3T3 and HeLa cells. In the examples which follow, CHO cells were utilized. The advantages thereof include the availability of suitable growth medium, their ability to grow efficiently and to high density in culture, and their ability to express mammalian proteins such as immunoglobulins in biologically active form.

Further, CHO cells were selected in large part because of previous usage of such cells by the inventors for the expression of immunoglobulins (using the translationally impaired dominant selectable marker containing vectors described previously). Thus, the present laboratory has considerable experience in using such cells for expression. However, based on the examples which follow, it is reasonable to expect similar results will be obtained with other mammalian cells.

In general, transformation or transfection of mammalian cells according to the subject invention will be effected according to conventional methods. So that the

invention may be better understood, the construction of exemplary vectors and their usage in producing integrants is described in the examples below.

EXAMPLE 1

5

Design and Preparation of Marker
and Targeting Plasmid DNA Vectors

The marker plasmid herein referred to as "Desmond" was assembled from the following DNA elements:

(a) Murine dihydrofolate reductase gene (DHFR),
10 incorporated into a transcription cassette, comprising the mouse beta globin promoter 5" to the DHFR start site, and bovine growth hormone poly adenylation signal 3" to the stop codon. The DHFR transcriptional cassette was isolated from TCAE6, an expression vector created
15 previously in this laboratory (Newman et al, 1992, *Bio-technology*, 10:1455-1460).

(b) E. coli β-galactosidase gene - commercially available, obtained from Promega as pSV-β-galactosidase control vector, catalog # E1081.

20 (c) Baculovirus DNA, commercially available, purchased from Clontech as pBAKPAK8, cat # 6145-1.

(d) Cassette comprising promoter and enhancer elements from Cytomegalovirus and SV40 virus. The cassette was generated by PCR using a derivative of expression
25 vector TCAE8 (Reff et al, *Blood*, 83:435-445 (1994)). The enhancer cassette was inserted within the baculo-

virus sequence, which was first modified by the insertion of a multiple cloning site.

(e) E. coli GUS (glucuronidase) gene, commercially available, purchased from Clontech as pB101, cat. #

5 6017-1.

(f) Firefly luciferase gene, commercially available, obtained from Promega as pGEM-Luc (catalog # E1541).

(g) S. typhimurium histidinol dehydrogenase gene (HisD). This gene was originally a gift from (Donahue et al, *Gene*, 18:47-59 (1982)), and has subsequently been incorporated into a transcription cassette comprising the mouse beta globin major promoter 5' to the gene, and the SV40 polyadenylation signal 3' to the gene.

15 The DNA elements described in (a)-(g) were combined into a pBR derived plasmid backbone to produce a 7.7kb contiguous stretch of DNA referred to in the attached figures as "homology". Homology in this sense refers to sequences of DNA which are not part of the mammalian genome and are used to promote homologous recombination between transfected plasmids sharing the same homology DNA sequences.

20 (h) Neomycin phosphotransferase gene from TN5 (Davis and Smith, *Ann. Rev. Micro.*, 32:469-518 (1978)).

25 The complete neo gene was subcloned into pBluescript SK- (Stratagene catalog # 212205) to facilitate genetic manipulation. A synthetic linker was then inserted into

a unique PstI site occurring across the codons for amino acid 51 and 52 of neo. This linker encoded the necessary DNA elements to create an artificial splice donor site, intervening intron and splice acceptor site within the neo gene, thus creating two separate exons, presently referred to as neo exon 1 and 2. Neo exon 1 encodes the first 51 amino acids of neo, while exon 2 encodes the remaining 203 amino acids plus the stop codon of the protein A NotI cloning site was also created within the 5 intron.

Neo exon 2 was further subdivided to produce neo exons 2 and 3. This was achieved as follows: A set of PCR primers were designed to amplify a region of DNA encoding neo exon 1, intron and the first 111 2/3 amino acids of exon2. The 3' PCR primer resulted in the introduction of a new 5' splice site immediately after the second nucleotide of the codon for amino acid 111 in exon 2, therefore generating a new smaller exon 2. The DNA fragment now encoding the original exon 1, intron and new exon 2 was then subcloned and propagated in a pBR based vector. The remainder of the original exon 2 was used as a template for another round of PCR amplification, which generated "exon3". The 5' primer for this round of amplification introduced a new splice acceptor site at the 5' side of the newly created exon 25 3, i.e. before the final nucleotide of the codon for amino acid 111. The resultant 3 exons of neo encode the

following information: exon 1 - the first 51 amino acids of neo; exon 2 - the next 111 2/3 amino acids, and exon 3 the final 91 1/3 amino acids plus the translational stop codon of the neo gene.

5 Neo exon 3 was incorporated along with the above mentioned DNA elements into the marking plasmid "Desmond". Neo exons 1 and 2 were incorporated into the targeting plasmid "Molly". The NotI cloning site created within the intron between exons 1 and 2 was used in 10 subsequent cloning steps to insert genes of interest into the targeting plasmid.

A second targeting plasmid "Mandy" was also generated. This plasmid is almost identical to "Molly" (some restriction sites on the vector have been changed) except that the original HisD and DHFR genes contained in "Molly" were inactivated. These changes were incorporated because the Desmond cell line was no longer being cultured in the presence of Histidinol, therefore it seemed unnecessary to include a second copy of the 20 HisD gene. Additionally, the DHFR gene was inactivated to ensure that only a single DHFR gene, namely the one present in the Desmond marked site, would be amplifiable in any resulting cell lines. "Mandy" was derived from "Molly" by the following modifications:

25 (i) A synthetic linker was inserted in the middle of the DHFR coding region. This linker created a stop codon and shifted the remainder of the DHFR coding

region out of frame, therefore rendering the gene nonfunctional.

(ii) A portion of the HisD gene was deleted and replaced with a PCR generated HisD fragment lacking the 5 promoter and start codon of the gene.

Figure 1 depicts the arrangement of these DNA elements in the marker plasmid "Desmond". Figure 2 depicts the arrangement of these elements in the first targeting plasmid, "Molly". Figure 3 illustrates the possible 10 arrangement in the CHO genome, of the various DNA elements after targeting and integration of Molly DNA into Desmond marked CHO cells. Figure 9 depicts the targeting plasmid "Mandy."

Construction of the marking and targeting plasmids 15 from the above listed DNA elements was carried out following conventional cloning techniques (see, e.g., Molecular Cloning, A Laboratory Manual, J. Sambrook et al, 1987, Cold Spring Harbor Laboratory Press, and Current Protocols in Molecular Biology, F. M. Ausubel et 20 al, eds., 1987, John Wiley and Sons). All plasmids were propagated and maintained in E. coli XLI blue (Stratagene, cat. # 200236). Large scale plasmid preparations were prepared using Promega Wizard Maxiprep DNA Purification System®, according to the 25 manufacturer's directions.

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EXAMPLE 2

Construction of a Marked CHO Cell Line

1. Cell Culture and Transfection Procedures to
Produced Marked CHO Cell Line

5 Marker plasmid DNA was linearized by digestion
overnight at 37°C with Bst1107I. Linearized vector was
ethanol precipitated and resuspended in sterile TE to a
concentration of 1mg/ml. Linearized vector was intro-
duced into DHFR-Chinese hamster ovary cells (CHO cells)
10 DG44 cells (Urlaub et al, *Som. Cell and Mol. Gen.*,
12:555-566 (1986)) by electroporation as follows.

Exponentially growing cells were harvested by cen-
trifugation, washed once in ice cold SBS (sucrose
buffered solution, 272mM sucrose, 7mM sodium phosphate,
15 pH 7.4, 1mM magnesium chloride) then resuspended in SBS
to a concentration of 10⁷ cells/ml. After a 15 minute
incubation on ice, 0.4ml of the cell suspension was
mixed with 40μg linearized DNA in a disposable
electroporation cuvette. Cells were shocked using a BTX
20 electrocell manipulator (San Diego, CA) set at 230
volts, 400 microfaraday capacitance, 13 ohm resistance.
Shocked cells were then mixed with 20 ml of prewarmed
CHO growth media (CHO-S-SFMII, Gibco/BRL, catalog #
31033-012) and plated in 96 well tissue culture plates.
25 Forty eight hours after electroporation, plates were fed
with selection media (in the case of transfection with
Desmond, selection media is CHO-S-SFMII without

hypoxanthine or thymidine, supplemented with 2mM Histidinol (Sigma catalog # H6647)). Plates were maintained in selection media for up to 30 days, or until some of the wells exhibited cell growth. These cells 5 were then removed from the 96 well plates and expanded ultimately to 120 ml spinner flasks where they were maintained in selection media at all times.

EXAMPLE 3

Characterization of Marked CHO Cell Lines

10 (a) Southern Analysis

Genomic DNA was isolated from all stably growing Desmond marked CHO cells. DNA was isolated using the Invitrogen Easy[®] DNA kit, according to the manufacturer's directions. Genomic DNA was then digested with 15 HindIII overnight at 37°C, and subjected to Southern analysis using a PCR generated digoxigenin labelled probe specific to the DHFR gene. Hybridizations and washes were carried out using Boehringer Mannheim's DIG easy hyb (catalog # 1603 558) and DIG Wash and Block 20 Buffer Set (catalog # 1585 762) according to the manufacturer's directions. DNA samples containing a single band hybridizing to the DHFR probe were assumed to be Desmond clones arising from a single cell which had integrated a single copy of the plasmid. These clones 25 were retained for further analysis. Out of a total of 45 HisD resistant cell lines isolated, only 5 were

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single copy integrants. Figure 4 shows a Southern blot containing all 5 of these single copy Desmond clones. Clone names are provided in the figure legend.

(b) Northern Analysis

5 Total RNA was isolated from all single copy Desmond clones using TRIzol reagent (Gibco/BRL cat # 15596-026) according to the manufacturer's directions. 10-20 μ g RNA from each clone was analyzed on duplicate formaldehyde gels. The resulting blots were probed with PCR generated digoxigenin labelled DNA probes to (i) DHFR message, (ii) HisD message and (iii) CAD message. CAD is a trifunctional protein involved in uridine biosynthesis (Wahl et al, *J. Biol. Chem.*, 254, 17:8679-8689 (1979)), and is expressed equally in all cell types. It is used here as an internal control to help quantitate RNA loading. Hybridizations and washes were carried out using the above mentioned Boehringer Mannheim reagents. The results of the Northern analysis are shown in Figure 5. The single copy Desmond clone exhibiting the highest levels of both the His D and DHFR message is clone 15C9, shown in lane 4 in both panels of the figure. This clone was designated as the "marked cell line" and used in future targeting experiments in CHO, examples of which are presented in the following sections.

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EXAMPLE 4

Expression of Anti-CD20 Antibody
in Desmond Marked CHO Cells

C2B8, a chimeric antibody which recognizes B-cell

5 surface antigen CD20, has been cloned and expressed previously in our laboratory. (Reff et al, *Blood*, 83:434-45 (1994)). A 4.1 kb DNA fragment comprising the C2B8 light and heavy chain genes, along with the necessary regulatory elements (eukaryotic promoter and polyadenylation signals) was inserted into the artificial 10 intron created between exons 1 and 2 of the neo gene contained in a pBR derived cloning vector. This newly generated 5kb DNA fragment (comprising neo exon 1, C2B8 and neo exon 2) was excised and used to assemble the targeting plasmid Molly. The other DNA elements used in 15 the construction of Molly are identical to those used to construct the marking plasmid Desmond, identified previously. A complete map of Molly is shown in Fig. 2.

The targeting vector Molly was linearized prior to 20 transfection by digestion with *Kpn*1 and *Pac*1, ethanol precipitated and resuspended in sterile TE to a concentration of 1.5mg/mL. Linearized plasmid was introduced into exponentially growing Desmond marked cells essentially as described, except that 80 μ g DNA was used in 25 each electroporation. Forty eight hours postelectroporation, 96 well plates were supplemented with selection medium - CHO-SSFMII supplemented with 400 μ g/mL Geneti-

cin (G418, Gibco/BRL catalog # 10131-019). Plates were maintained in selection medium for up to 30 days, or until cell growth occurred in some of the wells. Such growth was assumed to be the result of clonal expansion 5 of a single G418 resistant cell. The supernatants from all G418 resistant wells were assayed for C2B8 production by standard ELISA techniques, and all productive clones were eventually expanded to 120mL spinner flasks and further analyzed.

10 Characterization of Antibody secreting Targeted Cells

A total of 50 electroporations with Molly targeting plasmid were carried out in this experiment, each of which was plated into separate 96 well plates. A total of 10 viable, anti-CD20 antibody secreting clones were 15 obtained and expanded to 120ml spinner flasks. Genomic DNA was isolated from all clones, and Southern analyses were subsequently performed to determine whether the clones represented single homologous recombination events or whether additional random integrations had 20 occurred in the same cells. The methods for DNA isolation and Southern hybridization were as described in the previous section. Genomic DNA was digested with EcoRI and probed with a PCR generated digoxigenin labelled probe to a segment of the CD20 heavy chain constant 25 region. The results of this Southern analysis are presented in figure 6. As can be seen in the figure, 8 of

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the 10 clones show a single band hybridizing to the CD20 probe, indicating a single homologous recombination event has occurred in these cells. Two of the ten, clones 24G2 and 28C9, show the presence of additional band(s), indicative of an additional random integration elsewhere in the genome.

We examined the expression levels of anti-CD20 antibody in all ten of these clones, the data for which is shown in Table 1, below.

Table 1:

Expression Level of Anti-CD20
Secreting Homologous Integrants

	<u>Clone</u>	<u>Anti-CD20, pg/c/d</u>
	20F4	3.5
15	25E1	2.4
	42F9	1.8
	39G11	1.5
	21C7	1.3
	50G10	0.9
20	29F9	0.8
	5F9	0.3
	-----	-----
	28C9*	4.5
	24G2*	2.1

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5

* These clones contained additional randomly integrated copies of anti-CD20. Expression levels of these clones therefore reflect a contribution from both the homologous and random sites.

Expression levels are reported as picogram per cell per day (pg/c/d) secreted by the individual clones, and represented the mean levels obtained from three separate ELISAs on samples taken from 120 mL spinner flasks.

10 As can be seen from the data, there is a variation in antibody secretion of approximately ten fold between the highest and lowest clones. This was somewhat unexpected as we anticipated similar expression levels from all clones due to the fact the anti-CD20 genes are all
15 integrated into the same Desmond marked site. Nevertheless, this observed range in expression extremely small in comparison to that seen using any traditional random integration method or with our translationally impaired vector system.

20 Clone 20F4, the highest producing single copy integrant was selected for further study. Table 2 (below) presents ELISA and cell culture data from seven day production runs of this clone.

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Table 2:

7 Day Production Run Data for 20F4

Day	% Viable	viable/ml ($\times 10^5$)	T_{x2}(hr)	mg/L	pg/c/d
1	96	3.4	31	1.3	4.9
5	2	94	6	2.5	3.4
	3	94	9.9	33	4.7
	4	90	17.4	30	6.8
	5	73	14		8.3
	6	17	3.5		9.5

10

Clone 20F4 was seeded at 2×10^5 ml in a 120ml spinner flask on day 0. On the following six days, cell counts were taken, doubling times calculated and 1ml samples of supernatant removed from the flask and analyzed for secreted anti-CD20 by ELISA.

15

This clone is secreting on average, 3-5pg antibody/- cell/day, based on this ELISA data. This is the same level as obtained from other high expressing single copy clones obtained previously in our laboratory using the previously developed translationally impaired random integration vectors. This result indicates the following:

20

- (1) that the site in the CHO genome marked by the Desmond marking vector is highly transcriptionally active, and therefore represents an excellent site from which to express recombinant proteins, and

25

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(2) that targeting by means of homologous recombination can be accomplished using the subject vectors and occurs at a frequency high enough to make this system a viable and desirable alternative to random integration
5 methods.

To further demonstrate the efficacy of this system, we have also demonstrated that this site is amplifiable, resulting in even higher levels of gene expression and protein secretion. Amplification was achieved by plating serial dilutions of 20F4 cells, starting at a density of 2.5×10^4 cells/ml, in 96 well tissue culture dishes, and culturing these cells in media (CHO-SSFMII) supplemented with 5, 10, 15 or 20nM methotrexate. Antibody secreting clones were screened using standard ELISA
10 techniques, and the highest producing clones were expanded and further analyzed. A summary of this amplification experiment is presented in Table 3 below.
15

- 40 -

Table 3:

Summary of 20F4 Amplification

nM MTX	# Wells Assayed	Expression Level mg/l 96 well	# Wells Expanded	Expression Level pg/c/d from spinner
10	56	3-13	4	10-15
15	27	2-14	3	15-18
20	17	4-11	1	ND

Methotrexate amplification of 20F4 was set up as described in the text, using the concentrations of methotrexate indicated in the above table. Supernatants from all surviving 96 well colonies were assayed by ELISA, and the range of anti-CD20 expressed by these clones is indicated in column 3. Based on these results, the highest producing clones were expanded to 120ml spinners and several ELISAs conducted on the spinner supernatants to determine the pg/cell/day expression levels, reported in column 5.

The data here clearly demonstrates that this site can be amplified in the presence of methotrexate. Clones from the 10 and 15nM amplifications were found to produce on the order of 15-20pg/cell/day.

A 15nM clone, designated 20F4-15A5, was selected as the highest expressing cell line. This clone originated from a 96 well plate in which only 22 wells grew, and was therefore assumed to have arisen from a single cell.

A 15nM clone, designated 20F4-15A5, was selected as the highest expressing cell line. This clone originated

- 41 -

from a 96 well plate in which only 22 wells grew, and was therefore assumed to have arisen from a single cell. The clone was then subjected to a further round of methotrexate amplification. As described above, serial 5 dilutions of the culture were plated into 96 well dishes and cultured in CHO-SS-FMII medium supplemented with 200, 300 or 400nM methotrexate. Surviving clones were screened by ELISA, and several high producing clones were expanded to spinner cultures and further analyzed.

10 A summary of this second amplification experiment is presented in Table 4.

Table 4:
Summary of 20F4-15A5 Amplification

	nM MTX	# Wells Assayed	Expression Level mg/l 96 well	# Wells Expanded	Expression Level pg/c/d, spinner
15	200	67	23-70	1	50-60
	250	86	21-70	4	55-60
	300	81	15-75	3	40-50

20 Methotrexate amplifications of 20F4-15A5 were set up and assayed as described in the text. The highest producing wells, the numbers of which are indicated in column 4, were expanded to 120ml spinner flasks. The expression levels of the cell lines derived from these wells is recorded as pg/c/d in column 5.

25 The highest producing clone came from the 250nM methotrexate amplification. The 250nM clone, 20F4-15A5-250A6 originated from a 96 well plate in which only wells

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grew, and therefore is assumed to have arisen from a single cell. Taken together, the data in Tables 3 and 4 strongly indicates that two rounds of methotrexate amplification are sufficient to reach expression levels of 5 60pg/cell/day, which is approaching the maximum secretion capacity of immunoglobulin in mammalian cells (Reff, M.E., *Curr. Opin. Biotech.*, 4:573-576 (1993)). The ability to reach this secretion capacity with just 10 two amplification steps further enhances the utility of this homologous recombination system. Typically, random integration methods require more than two amplification steps to reach this expression level and are generally less reliable in terms of the ease of amplification. Thus, the homologous system offers a more efficient and 15 time saving method of achieving high level gene expression in mammalian cells.

EXAMPLE 5

Expression of Anti-Human CD23 Antibody in Desmond Marked CHO Cells

20 CD23 is low affinity IgE receptor which mediates binding of IgE to B and T lymphocytes (Sutton, B.J., and Gould, H.J., *Nature*, 366:421-428 (1993)). Anti-human CD23 monoclonal antibody 5E8 is a human gamma-1 monoclonal antibody recently cloned and expressed in our 25 laboratory. This antibody is disclosed in commonly

assigned Serial No. 08/803,085, filed on February 20, 1997.

The heavy and light chain genes of 5E8 were cloned into the mammalian expression vector N5KG1, a derivative 5 of the vector NEOSPLA (Barnett et al, in *Antibody Expression and Engineering*, H.Y Yang and T. Imanaka, eds., pp27-40 (1995)) and two modifications were then made to the genes. We have recently observed somewhat higher secretion of immunoglobulin light chains compared to 10 heavy chains in other expression constructs in the laboratory (Reff et al, 1997, unpublished observations). In an attempt to compensate for this deficit, we altered the 5E8 heavy chain gene by the addition of a stronger promoter/enhancer element immediately upstream of the 15 start site. In subsequent steps, a 2.9kb DNA fragment comprising the 5E8 modified light and heavy chain genes was isolated from the N5KG1 vector and inserted into the targeting vector Mandy. Preparation of 5E8-containing Molly and electroporation into Desmond 15C9 CHO cells 20 was essentially as described in the preceding section.

One modification to the previously described protocol was in the type of culture medium used. Desmond marked CHO cells were cultured in protein-free CD-CHO medium (Gibco-BRL, catalog # AS21206) supplemented with 25 3mg/L recombinant insulin (3mg/mL stock, Gibco-BRL, catalog # AS22057) and 8mM L-glutamine (200mM stock, Gibco-BRL, catalog # 25030-081). Subsequently, trans-

fected cells were selected in the above medium supplemented with 400 μ g/mL geneticin. In this experiment, 20 electroporations were performed and plated into 96 well tissue culture dishes. Cells grew and secreted anti-5 CD23 in a total of 68 wells, all of which were assumed to be clones originating from a single G418 cell. Twelve of these wells were expanded to 120ml spinner flasks for further analysis. We believe the increased number of clones isolated in this experiment (68 compared with 10 for anti-CD20 as described in Example 4) is due to a higher cloning efficiency and survival rate 10 of cells grown in CD-CHO medium compared with CHO-SS-FMII medium. Expression levels for those clones analyzed in spinner culture ranged from 0.5-3pg/c/d, in close agreement with the levels seen for the anti-CD20 15 clones. The highest producing anti-CD23 clone, designated 4H12, was subjected to methotrexate amplification in order to increase its expression levels. This amplification was set up in a manner similar to that described 20 for the anti-CD20 clone in Example 4. Serial dilutions of exponentially growing 4H12 cells were plated into 96 well tissue culture dishes and grown in CD-CHO medium supplemented with 3mg/L insulin, 8mM glutamine and 30, 35 or 40nM methotrexate. A summary of this 25 amplification experiment is presented in Table 5.

Table 5:

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Summary of 2H12 Amplification

nM MTX	# Wells Assayed	Expression Level mg/l 96 well	# Wells Expanded	Expression Level pg/c/d from spinner
30	100	6-24	8	10-25
35	64	4-27	2	10-15
5	40	4-20	1	ND

The highest expressing clone obtained was a 30nM clone, isolated from a plate on which 22 wells had grown. This clone, designated 4H12-30G5, was reproducibly secreting 18-22pg antibody per cell per day. This is the same range of expression seen for the first amplification of the anti CD20 clone 20F4 (clone 20F4-15A5 which produced 15-18pg/c/d, as described in Example 4). This data serves to further support the observation that amplification at this marked site in CHO is reproducible and efficient. A second amplification of this 30nM cell line is currently underway. It is anticipated that saturation levels of expression will be achievable for the anti-CD23 antibody in just two amplification steps, as was the case for anti-CD20.

20

EXAMPLE 6

Expression of Immunoadhesin in Desmond Marked CHO Cells

CTLA-4, a member of the Ig superfamily, is found on the surface of T lymphocytes and is thought to play a role in antigen-specific T-cell activation (Dariavach et al, *Eur. J. Immunol.*, 18:1901-1905 (1988); and Linsley et al, *J. Exp. Med.*, 174:561-569 (1991)). In order to further study the precise role of the CTLA-4 molecule in the activation pathway, a soluble fusion protein comprising the extracellular domain of CTLA-4 linked to a truncated form of the human IgG1 constant region was

created (Linsley et al (Id.)). We have recently expressed this CTLA-4 Ig fusion protein in the mammalian expression vector BLECH1, a derivative of the plasmid NEOSPLA (Barnett et al, in Antibody Expression and Engineering, H.Y Yang and T. Imanaka, eds., pp27-40 (1995)). An 800bp fragment encoding the CTLA-4 Ig was isolated from this vector and inserted between the SacII and BglII sites in Molly.

Preparation of CTLA-4Ig-Molly and electroporation into Desmond clone 15C9 CHO cells was performed as described in the previous example relating to anti-CD20. Twenty electroporations were carried out, and plated into 96 well culture dishes as described previously. Eighteen CTLA-4 expressing wells were isolated from the 96 well plates and carried forward to the 120ml spinner stage. Southern analyses on genomic DNA isolated from each of these clones were then carried out to determine how many of the homologous clones contained additional random integrants. Genomic DNA was digested with BglII and probed with a PCR generated digoxigenin labelled probe to the human IgG1 constant region. The results of this analysis indicated that 85% of the CTLA-4 clones are homologous integrants only; the remaining 15% contained one additional random integrant. This result corroborates the findings from the expression of anti-CD20 discussed above, where 80% of the clones were single homologous integrants. Therefore, we can conclude

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that this expression system reproducibly yields single targeted homologous integrants in at least 80% of all clones produced.

Expression levels for the homologous CTLA4-Ig
5 clones ranged from 8-12 pg/cell/day. This is somewhat higher than the range reported for anti-CD20 antibody and anti-CD23 antibody clones discussed above. However, we have previously observed that expression of this molecule using the intronic insertion vector system also
10 resulted in significantly higher expression levels than are obtained for immunoglobulins. We are currently unable to provide an explanation for this observation.

EXAMPLE 7

Targeting Anti-CD20 to an alternate Desmond Marked CHO Cell Line

As we described in a preceding section, we obtained
5 single copy Desmond marked CHO cell lines (see Figures
4 and 5). In order to demonstrate that the success of
our targeting strategy is not due to some unique proper-
ty of Desmond clone 15C9 and limited only to this clone,
we introduced anti-CD20 Molly into Desmond clone 9B2
(lane 6 in figure 4, lane 1 in figure 5). Preparation
of Molly DNA and electroporation into Desmond 9B2 was
exactly as described in the previous example pertaining
20 to anti-CD20. We obtained one homologous integrant from
this experiment. This clone was expanded to a 120ml
25

spinner flask, where it produced on average 1.2pg anti-CD20/cell/day. This is considerably lower expression than we observed with Molly targeted into Desmond 15C9. However, this was the anticipated result, based on our 5 northern analysis of the Desmond clones. As can be seen in Figure 5, mRNA levels from clone 9B2 are considerably lower than those from 15C9, indicating the site in this clone is not as transcriptionally active as that in 15C9. Therefore, this experiment not only demonstrates 10 the reproducibility of the system - presumably any marked Desmond site can be targeted with Molly - it also confirms the northern data that the site in Desmond 15C9 is the most transcriptionally active.

From the foregoing, it will be appreciated that, 15 although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without diverting from the scope of the invention. Accordingly, the invention is not limited by the appended claims.

WHAT IS CLAIMED IS:

1. A method for inserting a desired DNA at a target site in the genome of a mammalian cell which comprises the following steps:

5 (i) transfected or transforming a mammalian cell with a first plasmid ("marker plasmid") containing the following sequences:

(a) a region of DNA that is heterologous to the mammalian cell genome which when integrated in the 10 mammalian cell genome provides a unique site for homologous recombination;

(b) a DNA fragment encoding a portion of a first selectable marker protein; and

15 (c) at least one other selectable marker DNA that provides for selection of mammalian cells which have been successfully integrated with the marker plasmid;

(ii) selecting a cell which contain the marker plasmid integrated in its genome;

20 (iii) transfected or transforming said selected cell with a second plasmid ("target plasmid") which contains the following sequences:

(a) a region of DNA that is identical or is sufficiently homologous to the unique region in the 25 marker plasmid such that this region of DNA can recombine with said DNA via homologous recombination;

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5 (b) a DNA fragment encoding a portion of the same selectable marker contained in the marker plasmid, wherein the active selectable marker protein encoded by said DNA is only produced if said fragment is expressed in association with the fragment of said selectable marker DNA contained in the marker plasmid; and

 (iv) selecting cells which contain the target plasmid integrated at the target site by screening for the expression of the first selectable marker protein.

10 2. The method of Claim 1, wherein the DNA fragment encoding a fragment of a first selectable marker is an exon of a dominant selectable marker.

15 3. The method of Claim 2, wherein the second plasmid contains the remaining exons of said first selectable marker.

4. The method of Claim 3, wherein at least one DNA encoding a desired protein is inserted between said exons of said first selectable marker contained in the target plasmid.

20 5. The method of Claim 4, wherein a DNA encoding a dominant selectable marker is further inserted between the exons of said first selectable marker contained in

the target plasmid to provide for co-amplification of the DNA encoding the desired protein.

6. The method of Claim 3, wherein the first dominant selectable marker is selected from the group consisting of neomycin phosphotransferase, histidinol dehydrogenase, dihydrofolate reductase, hygromycin phosphotransferase, herpes simplex virus thymidine kinase, adenosine deaminase, glutamine synthetase, and hypoxanthine-guanine phosphoribosyl transferase.

10 7. The method of Claim 4, wherein the desired protein is a mammalian protein.

8. The method of Claim 7, wherein the protein is an immunoglobulin.

15 9. The method of Claim 1, which further comprises determining the RNA levels of the selectable marker (c) contained in the marker plasmid prior to integration of the target vector.

20 10. The method of Claim 9, wherein the other selectable marker contained in the marker plasmid is a dominant selectable marker selected from the group consisting of histidinol dehydrogenase, herpes simplex

thymidine kinase, hydromycin phosphotransferase, adenosine deaminase and glutamine synthetase.

11. The method of Claim 1, wherein the mammalian cell is selected from the group consisting of Chinese hamster ovary (CHO) cells, myeloma cells, baby hamster kidney cells, COS cells, NSO cells, HeLa cells and NIH 3T3 cells.

12. The method of Claim 11, wherein the cell is a CHO cell.

10 13. The method of Claim 1, wherein the marker plasmid contains the third exon of the neomycin phosphotransferase gene and the target plasmid contains the first two exons of the neomycin phosphotransferase gene.

15 14. The method of Claim 1, wherein the marker plasmid further contains a rare restriction endonuclease sequence which is inserted within the region of homology.

20 15. The method of Claim 1, wherein the unique region of DNA that provides for homologous recombination is a bacterial DNA, a viral DNA or a synthetic DNA.

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16. The method of Claim 1, wherein the unique region of DNA that provides for homologous recombination is at least 300 nucleotides.

5 17. The method of Claim 16, wherein the unique region of DNA ranges in size from about 300 nucleotides to 20 kilobases.

18. The method of claim 17, wherein the unique region of DNA preferably ranges in size from 2 to 10 kilobases.

10 19. The method of Claim 1, wherein the first selectable marker DNA is split into at least three exons.

15 20. The method of Claim 1, wherein the unique region of DNA that provides for homologous recombination is a bacterial DNA, an insect DNA, a viral DNA or a synthetic DNA.

21. The method of Claim 20, wherein the unique region of DNA does not contain any functional genes.

20 22. A vector system for inserting a desired DNA at a target site in the genome of a mammalian cell which comprises at least the following:

(i) a first plasmid ("marker plasmid") containing at least the following sequences:

(a) a region of DNA that is heterologous to the mammalian cell genome which when integrated in the mammalian cell genome provides a unique site for homologous recombination;

(b) a DNA fragment encoding a portion of a first selectable marker protein; and

(c) at least one other selectable marker DNA that provides for selection of mammalian cells which have been successfully integrated with the marker plasmid; and

(ii) a second plasmid ("target plasmid") which contains at least the following sequences:

(a) a region of DNA that is identical or is sufficiently homologous to the unique region in the marker plasmid such that this region of DNA can recombine with said DNA via homologous recombination;

(b) a DNA fragment encoding a portion of the same selectable marker contained in the marker plasmid, wherein the active selectable marker protein encoded by said DNA is only produced if said fragment is expressed in association with the fragment of said selectable marker DNA contained in the marker plasmid.

23. The vector system of Claim 22, wherein the DNA fragment encoding a fragment of a first selectable marker is an exon of a dominant selectable marker.

24. The vector system of Claim 23, wherein the
5 second plasmid contains the remaining exons of said
first selectable marker.

25. The vector system of Claim 24, wherein at
least one DNA encoding a desired protein is inserted
between said exons of said first selectable marker con-
10 tained in the target plasmid.

26. The vector system of Claim 24, wherein a DNA
encoding a dominant selectable marker is further insert-
ed between the exons of said first selectable marker
contained in the target plasmid to provide for co-ampli-
15 fication of the DNA encoding the desired protein.

27. The vector system of Claim 24, wherein the
first dominant selectable marker is selected from the
group consisting of neomycin phosphotransferase,
histidinol dehydrogenase, dihydrofolate reductase,
20 hygromycin phosphotransferase, herpes simplex virus
thymidine kinase, adenosine deaminase, glutamine synthe-
tase, and hypoxanthine-guanine phosphoribosyl transfer-
ase.

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28. The vector system of Claim 25, wherein the desired protein is a mammalian protein.

29. The vector system of Claim 28, wherein the protein is an immunoglobulin.

5 30. The vector system of Claim 22, wherein the other selectable marker contained in the marker plasmid is a dominant selectable marker selected from the group consisting of histidinol dehydrogenase, herpes simplex thymidine kinase, hydromycin phosphotransferase, adenosine deaminase and glutamine synthetase.

10 31. The vector system of Claim 22, which provides for insertion of a desired DNA at a targeted site in the genome of a mammalian cell selected from the group consisting of Chinese hamster ovary (CHO) cells, myeloma cells, baby hamster kidney cells, COS cells, NSO cells, HeLa cells and NIH 3T3 cells.

15 32. The vector system of Claim 31, wherein the mammalian cell is a CHO cell.

20 33. The vector system of Claim 22, wherein the marker plasmid contains the third exon of the neomycin phosphotransferase gene and the target plasmid contains

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the first two exons of the neomycin phosphotransferase gene.

34. The vector system of Claim 22, wherein the marker plasmid further contains a rare restriction endonuclease sequence which is inserted within the region of homology.

5
35. The vector system of Claim 22, wherein the unique region of DNA that provides for homologous recombination is a bacterial DNA, a viral DNA or a synthetic DNA.

10
36. The vector system of Claim 22, wherein the unique region of DNA (a) contained in the marker plasmid vector system that provides for homologous recombination is at least 300 nucleotides.

15
37. The vector system of Claim 36, wherein the unique region of DNA ranges in size from about 300 nucleotides to 20 kilobases.

20
38. The vector system of Claim 37, wherein the unique region of DNA preferably ranges in size from 2 to 10 kilobases.

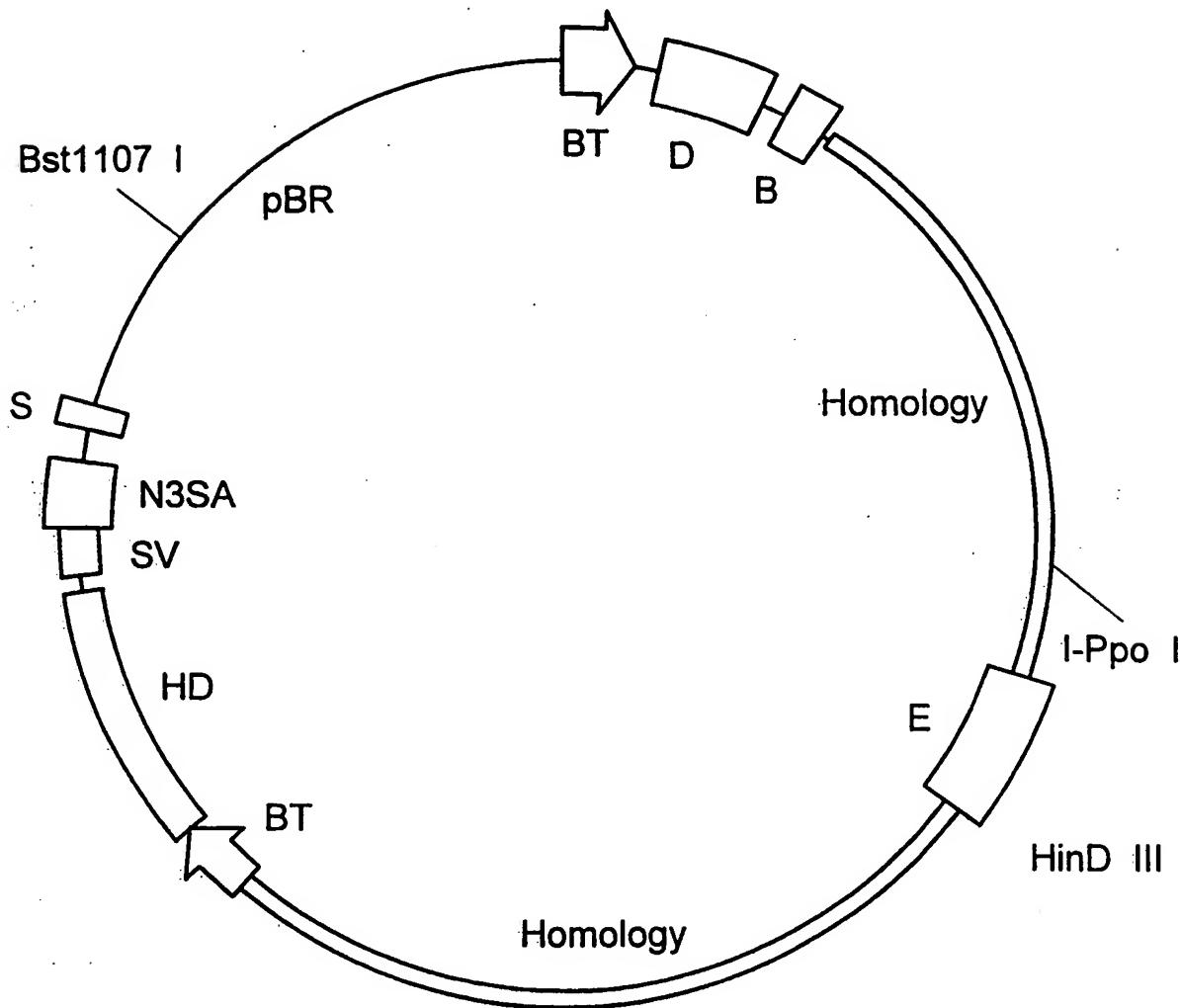
- 58 -

39. The vector system of Claim 22, wherein the first selectable marker DNA is split into at least three exons.

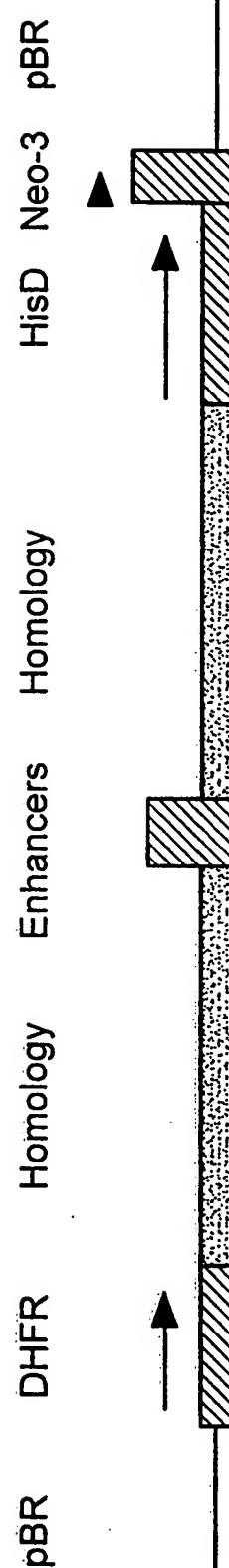
40. The vector system of Claim 22, wherein the
5 unique region of DNA that provides for homologous recombination is a bacterial DNA, an insect DNA, a viral DNA or a synthetic DNA.

41. The vector system of Claim 40, wherein the
10 unique region of DNA does not contain any functional genes.

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**FIG. 1A
DESMOND**HD = *Salmonella HisD Gene*N3 = *Neomycin Phosphotransferase Exon 3*D = *Murine Dihydrofolate reductase*E = *Cytomegalovirus and SV40 Enhancers*SA = *Splice acceptor*BT = *Mouse Beta Globin Major Promoter*B = *Bovine Growth Hormone Polyadenylation*S = *SV40 Early Polyadenylation*SV = *SV40 Late Polyadenylation*

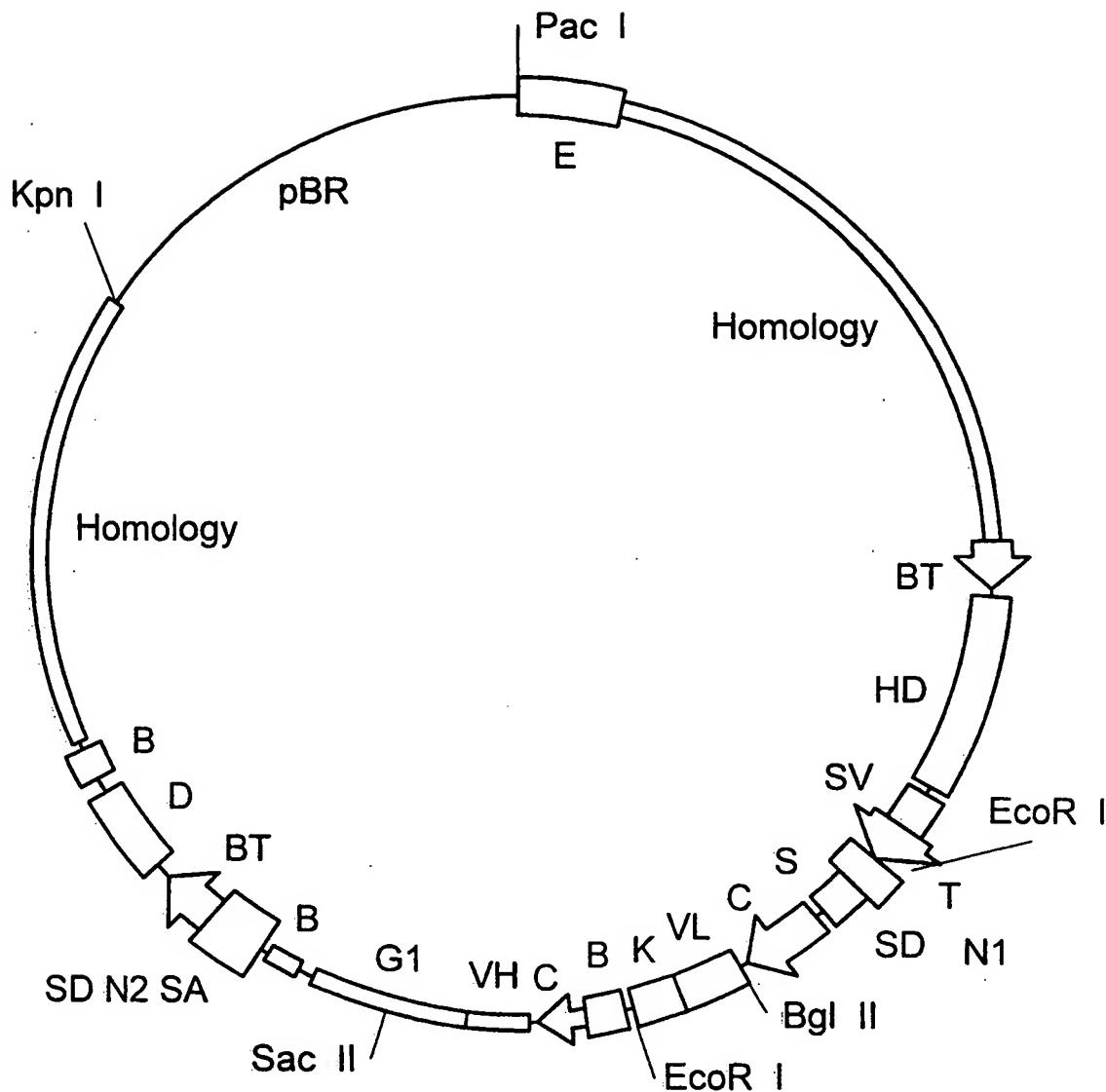
2/75

Desmond**14,683 bp Bst1107 I linear****FIG. 1B**

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Molly

FIG. 2A



D = Dihydrofolate reductase

N1 + Neomycin Phosphotransferase Exon 1

N2 + Neomycin Phosphotransferase Exon 2

VL = Anti-CD20 Light chain leader + Variable

K = Human Kappa Constant

VH = Anti-CD20 Heavy chain Leader + Variable

G1 = Human Gamma 1 Constant

HD = Salmonella Histidinol Dehydrogenase

E = CMV and SV40 enhancers S = SV40 Origin

SD = Splice donor SA = Splice acceptor

C = CMV promoter/enhancer

T = HSV TK promoter and Poloma enhancers

BT = Mouse Beta Globin Major Promoter

SV = SV40 Late Polyadenylation

B = Bovine Growth Hormone Polyadenylation

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Molly
15,987 bp Pac I, Kpn I fragment

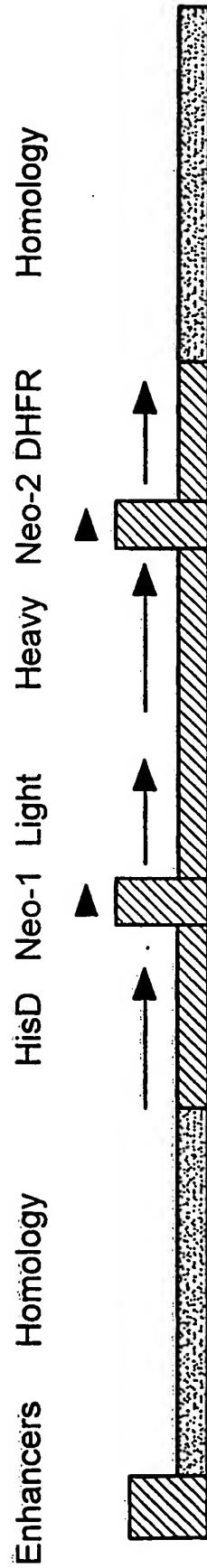
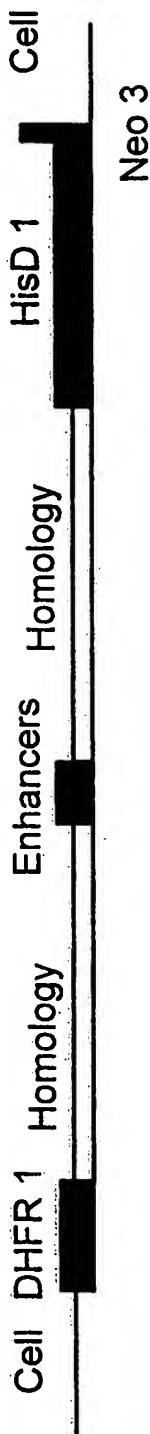


FIG. 2B

FIG. 3

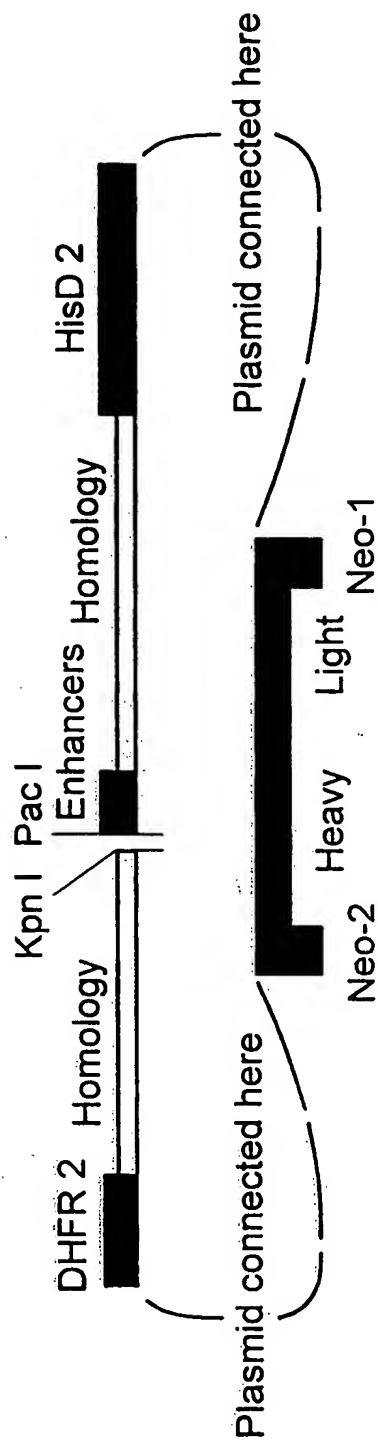
Homologous Recombination

Desmond in CHO



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Molly



Single crossover in CHO



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Southern Analysis of Desmond Marked CHO Cells

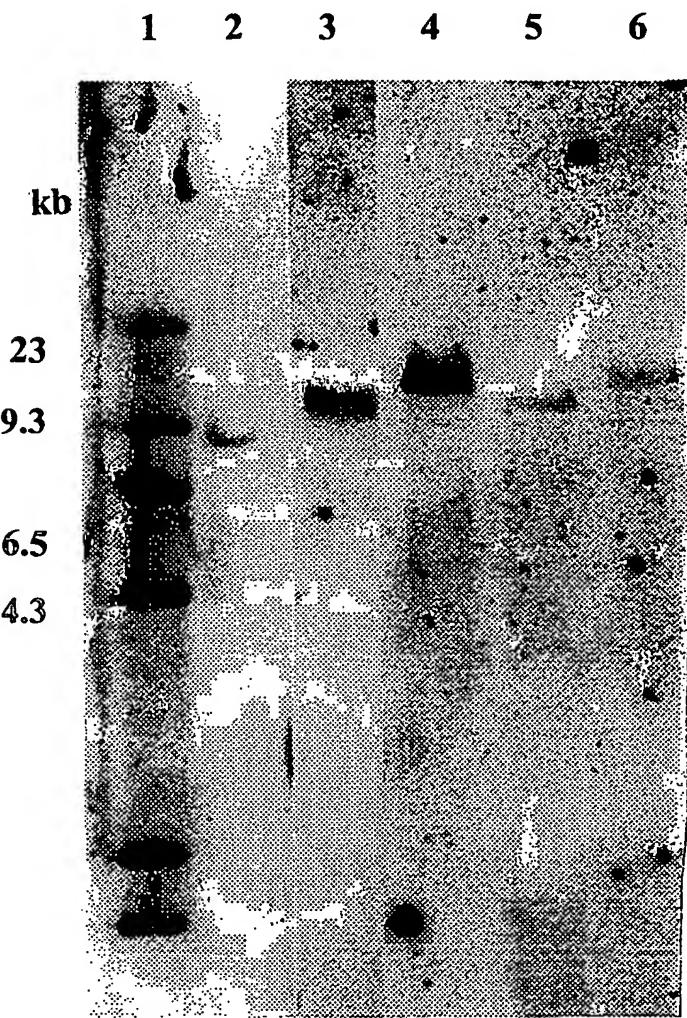


FIG. 4

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Northern Analysis of Desmond Marked CHO Cells

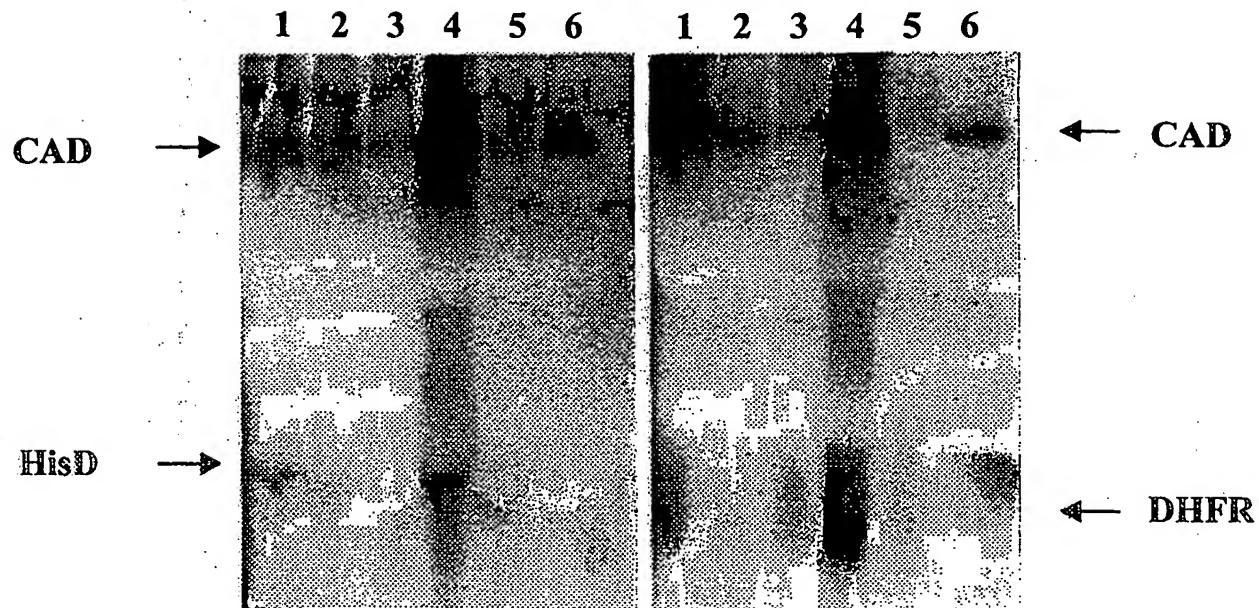


FIG. 5

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Southern Analysis of Anti CD20 Integrants in Marked CHO Cells

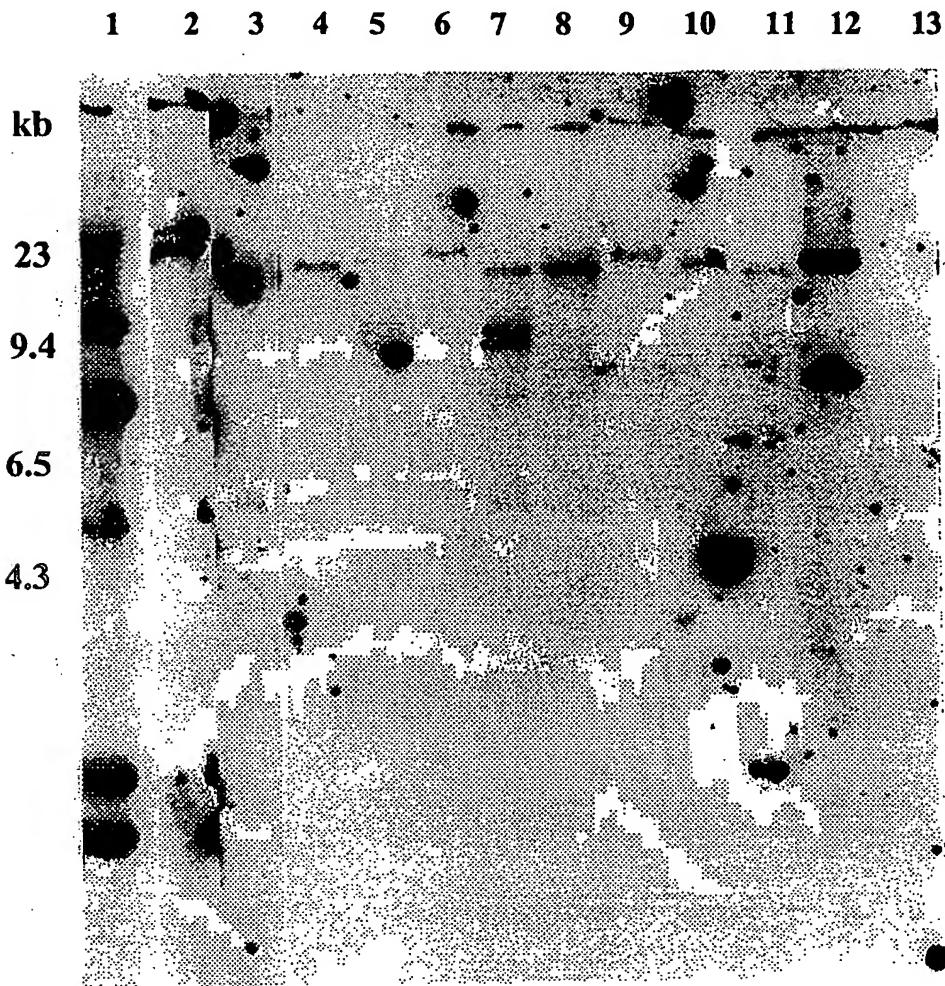


FIG. 6

FIG. 7A

TTCTAGACC TAGGGCGGCC AGCTAGTAGC TTTGCTTCTC AATTTCCTTAT TTGCATAATG
60
AGAAAAAAAG GAAAATTAAAT TTTAACACCCA ATTCACTAGT TGATTGAGCA AATGCGTTG
120
CAAAAGGAT GCTTTAGAGA CAGTGTTCTC TGCACAGATA AGGACAAACA TTATTCAAGAG
180
GGAGTACCCA GAGCTGAGAC TCCTAAGCCA GTGAGTGGCA CAGCATTCTA GGGAGAAATA
240
TGCTTGTCA CACCGAAGGCC TGATTCCGTA GAGCCACACC TTGGTAAGGG CCAAATCTGCT
300
CACACAGGAT AGAGAGGGCA GGAGGCCAGGG CAGAGGCATAT AAGGGTGAGGT AGGATCAAGT
360
GCTCCTCACA TTTGCTTCTG ACATAGTTGT GTTGGGAGGCT TGATAGCTT GGACAGGCTCA
420
GGGCTGCGAT TTCGGCCAA ACTTGACGGC AATCCTAGCG TGAAGGGCTGG TAGGATTTTA
480
TCCCCGCTGC CATCATGGTT CGACCATGTA ACTGCATCGT CGCCGTGTCC CAAAATATGG
540
GGATTGGCAA GAACGGAGAC CTACCCCTGGC CTCCGCTCAG GAACGGAGTTC AAGTACTTCC
600
AAAGAATGAC CACAACCTCT TCAGTGGAG GTAAACAGAA TCTGGTGATT ATGGGTAGGA
660

FIG. 7B

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AAACCTGGTT CTCCATTCT GAGAAGAACATC GACCCTTAAA GGACAGAAATT AATATAAGTTTC
720
TCAGTAGAGA ACTCAAAGAA CCACCAACGAG GAGGCTCATT TCTTGCCTAA AGTTGGATG
780
ATGCCCTTAAG ACTTATTGAA CAACCGGAAT TGGCAAGTAA AGTAGACATG GTTTGGATAG
840
TCGGAGGCAG TTCTGTTAC CAGGAAGCCA TGAATCAACC AGGCCACCTT AGACTCTTTG
900
TGACAAGGAT CATGCAGGAA TTTGAAAGTG ACACGTTTT CCCAGAAATT GATTGGGGAA
960
AATATAACT TCTCCAGAA TACCCAGGCG TCCTCTCTGA GGTCCAGGAG GAAAAGGCA
1020
TCAAGTATAA GTTTGAAGTC TAGGAGAAGA AAGACTAACAA GGAAGATGCT TTCAAGTTCT
1080
CTGCTCCCT CCTAAAGCTA TGCATTTTTA TAAGACCATG GGACCTTTGC TGGCTTTAGA
1140
TCAGCCTCGA CTGTGCCTTC TAGTTGCCAG CCATCTGTTG TTTGCCCTC CCCCCTGCCT
1200
TCCCTGACCC TGGAAAGGTGC CACTCCCCACT GTCCCTTCCCT AATAAAATGAA GGAAATTGCA
1260
TCGCATTGTC TGAGTAGGTG TCATTCTATT CTGGGGGGTG GGGTGGGGCA GGACAGCAAG
1320

FIG. 7C

GGGGAGGATT GGGAAAGACAA TAGCAGGCAT GCTGGGGATG CGGTGGGCTC TATGGAAACCA
1380
GCTGGGGCTC GAAGCGGGCC CCCATTTCGG CCCATTTCGG TGGTGGTCAG ATGGGGGATG GCGTGGGACGG
1440
CGGGGGGAC CGTCACACTG AGGTTTCCG CCAGACGCCA CTGCTGCCAG GCGCTGATGT
1500
GCCCGGGCTC TGACCATGCG GTCGCGTTCG GTTGCAC TAC GCGTACTGTG AGCCAGAGGTT
1560
GCCCGGGCT CTCGGCTGC GGTAGTTCAAG GCAGTTCAAT CAACTGTTA CCTTGTTGAG
1620
CGACATCCAG AGGCACCTCA CCGGCTTGCTA GCGGCTTACC ATCCAGGCC ACCATCCAGT
1680
GCAGGAGCTC GTTATCGCTA TGACGGAAACA GGTATTTCGCT GGTCACTTCG ATGGTTGCC
1740
CGGATAAACG GAACTGGAAA AACTGCTGCT GGTGTTTGC TTCCGGTCAGC GCTGGATGCG
1800
GCGTGGCTC GGCAAAGACC AGACCGTTCA TACAGAACTG GCGATCGTTC GGCGTATCAC
1860
CAAATCACC GCCGTAAGCC GACCACGGGT TGCCGTTTC ATCATATTTA ATCAGCGACT
1920
GATCCACCCA GTCCAGACG AAGCCGCCCT GTAAACGGGG ATACTGACGA AACGCCCTGCC
1980

FIG. 7D

AGTATTTAGC GAAACCGCCA AGACTGTTAC CCATCGCGTG GGCGTATTTCG CAAAGGGATCA
GGGGGGCGT CTCTCCGGGT AGCGAAAGGCC ATTTCGGTGGT GGACCATTTC GGACCCAGGCC
GGAAAGGGCTG GTCTCATCC ACGCGGGCGT ACATCGGGCA AATAATATCG GTGGCCGTGG
TGTCTGCC GCCGGCTTC TACTGCACCG GCGGGGAAGG ATCGACAGAT TTGATCCAGC
GATACAGCGC GTCGTGATTAA GCGCCGTGGC CTGATTCACTT CCCCAGCGAC CAGATGATCA
CACTCGGGTG ATTACGATCG CGCTGCACCA TTTCGGTGTAC GCCTTCGCTC ATCGCCGGTA
GCCAGCGGG ATCATCGGTC AGACGATTCA TTGGCACCAT GCCGTGGTT TCAATATTGG
CTTCATCCAC CACATACAGG CGTAGCGGT CGCACAGCGT GTACCCACAGGC GGATGGTTCG
GATAATGCGA ACAGCGCAGC GCGTTAAAGT TGTTCGTGCTT CATCAGCAGG ATATCCCTGCA
CCATCGTCTG CTCATCCATG ACCTGACCAT GCAGGGATG ATGCTCGTGA CGGTTAACGC
CTCGAATCAG CAACGGCTTG CCGTTCAGCA GCAGCAGGACCC ATTTCAAATC CGCACCTCGC
12/75
2040 2100 2160 2220 2280 2340 2400 2460 2520 2580 2640

FIG. 7E

GGAAACCGAC ATCGCAGGCT TCTGCCCAA TCAGCGTGTGCC GTCGGCGGTG TGCAGTTCAA
CCACCGCACG ATAGAGATTTC GGGATTTCGG CGCTCCACAG TTTCGGGTTT TCGACCGTTCA
2700
GACGGCAGTGT GACGGCGATCG GCATAACCAC CAGGCTCATC GATAATTTCAC CGGCCGAAAG
2760
GCCGGGTGCC GCTGGCGACC TGCGTTTCAC CCTGCCATAA AGAAACTGTT ACCCGTAGGT
2820
AGTCACGCCAA CTCGCCGCAC ATCTGAACCT CAGCCTCCAG TACAGCGGG CTGAAATCAT
2880
CATTAAGCG AGTGGCAACA TGAAATCGC TGATTTGTGT AGTGGGTTA TGCAGCAACG
2940
AGACGTCACG GAAATGCCG CTCATCGGC ACATATCCTG ATCTTCCAGA TAACTGCCGT
3000
CACTCCACG CAGCACCATC ACCGCCGAGGC GGTTTTCTCC GGCGCGTAA AATGCCGCTCA
3060
GGTCAAATTTC AGACGGCAA CGACTGTCCCT GGCTGTAAACC GACCCACGCC CGTTGCACC
3120
ACAGATGAAA CGCCCGAGTTA ACGCCATCAA AAATAATTTCG CGTCTGGCCT TCCTGTAGCC
3180
AGCTTTCATC AACATTAAAT GTGAGCGAGT AACAAACCCGT CGGATTCTCC GTGGGAACAA
3240
3300

FIG. 7F

ACGGCGGATT GACCGTAATG GGATAGGTTA CGTTGGTGTAA GATGGGCCA TCGTAACCGT
GCATCTGCCA GTTGGGG ACCGACG TATCGGCCTC AGGAAGATCG CACTCCAGCC 3360
AGCTTTCCGG CACTGCTTCT GGTGCCGGAA ACCAGGCAA GCGCCATTTCG CCATTCAAGGC 3420
TGCGCAACTG TTGGGAAGGG CGATCGGTGC GGCCCTCTTC GCTATTACGC CAGCTGGCGA 3480
AAGCGGGATG TGCTGCAAGG CGATTAAGTT GGGTAACGCC AGGGTTTTCC CAGTCACGAC 3540
GTTGTAAAAC GACTTAATCC GTCGAGGGGC TGCTCGAAG CAGACGACCT TCCGTTGTGC 3600
AGCCAGCGGC GCCTGCGCC GTGCCACAA TCGTGGCGA ACAAACTAA CCAGAACAAA 3660
TCATACGGC GGCACCCGG CCACCAACCTT CTCTGTGCC TAACATTCCA GCGCCTCCAC 3720
CACTACCACC ACCATCGATG TCTGAATTGC CGCCCCGCTCC ACCAATGCCG ACGGAACCTC 3780
AACCCGCTGC ACCTTTAGAC GACAGACAAC AATTGTTGGA AGCTATTAGA AACGAAAAAA 3840
ATCGCACCTCG TCTCAGACCG GCTCTTAA GGTAGCTCAA ACCAAAAACG GCGCCCCGAAA 3900
3960

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FIG. 7G

CCAGTACAAT AGTTGAGGTG CCGACTGTGT TGCCTAAAGA GACATTTGAG CTTAAACCGC
CGTCTGCACC ACCGCCACCA CCTCCGGCTC CGCCTCCAC GCCAGCCCCC CCTGCCGCCTC
4020
CACCGATGGT AGATTCAATCA TCAGCTCCAC CACCGCCGCC ATTAGTAGAT TTGCCGGTCTG
4080
AAATGTTACC ACCGGCCTGCA CCATCGCTTT CTAACGTGTT GTCTGAATTAA AAATCGGGCA
4140
CAGTTAGATT GAAACCCGCC CAAAAACGCC CGCAATCAGA AATAATTCCA AAAAGCTCAA
4200
CTACAAATTGATCGGGAC GTGTTAGGCC ACACAAATTAA TAGGCGTCGT GTGGCTATGG
4260
CAAATCGTC TTGGGAAGCA ACTTCTAACG ACGAGGGTTG GGACGGACGAC GATAATCGGC
4320
CTATAAAAGC TAACACGCC GATGTTAAAT ATGTCCAAGC TACTAGTGGT ACCTTAATTAA
4380
AGGGGGAG AATGGGGGA ACTGGGGGA GTTAGGGCG GGATGGGG AGTTAGGGGC
4440
GGGACTATGG TTGCTGACTA ATTGAGATGC ATGCTTTGCA TACTTCTGCC TGCTGGGAG
4500
CCTGGGGACT TTCCACACCT GGTGCTGAC TAATTGAGAT GCATGCTTGC CATACTTCTG
4560
4620

FIG. 7H

CCTGCTGGG AGCCCTGGGA CTTTCCAC CCTTAAC TGAC ACACATTCCA CAGAATTAAAT
TCCCCTAGTT ATTAAATAGTA ATCAATTACG GGGTCATTAG TTTCATAGCCC ATATATGGAG 4680
TTCCGCCTTA CATAACTTAC GGTAATGGC CCCCTGGCT GACCGCTCAA CGACCCCCGC 4740
CCATTGACGT CAATAATGAC GTATGTTCCC ATAGTAACGCC CAATAGGGAC TTTCCATTGA 4800
CGTCAATGGG TGGACTATT ACGGTAAACT GCCCACTTGG CAGTACATCA AGTGTATCAT 4860
ATGCCAAGTA CGCCCCCTAT TGACGTCAAAT GACGGTAAAT GGCCCCGCCTG GCATTATGCC 4920
CAGTACATGA CCTTATGGGA CTTTCCTACT TGGCAGTACA TCTACGTATT AGTCATCGCT 4980
ATTACCATGG TGATGCGGTT TTGGCAGTAC ATCAATGGGC GTGGATAGGC GTTTGACTCA 5040
CGGGGATTTC CAAGTCTCCA CCCCATTGAC GTCAATGGGA GTTTGTTTG AAGCTTGGCC 5100
GCCCATATAA ACGGCGGCCA GCTTTATTTA ACGTGTAC GTCGAGTCAA TTGTACACTA 5160
ACGACAGTGA TGAAAGAAAT ACAAAAGCGC ATAATATTT GAACGGACGTC GAACCTTTAT 5220
5280

16/75

FIG. 71

TACAAACAA AACACAAACG AATATCGACA AAGCTAGATT GCTGCTACAA GATTTGGCAA
5340
GTTTGTGGC GTTGAGGGAA AATCCATTAG ATAGTCCAGC CATCGGGTCG GAAAAACAAAC
5400
CCTTGTGTGA AACTAATCGA AACCTATTT ACAAAATCTAT TGAGGGATTAA ATATTTAAAT
5460
TCAGATATAA AGACGGCTGAA AATCATTGAA TTTTCGCTCT AACATACCAC CCTAAAGATT
5520
ATAAATTAA TGAATTATA AAATACATCA GCAACTATAT ATTGATAGAC ATTCCAGTT
5580
TGTGATATAA GTTTGTGGT CTCATTACAA TGGCTGTTAT TTTTAACAAAC AACAAACTGC
5640
TCGCAGACAA TAGTATAGAA AAGGGAGGGTG AACTGTTTT GTTAAACGGT TCGTACAAACA
5700
TTTTGGAAAG TTATGTTAAT CCGGTGCTGC TAAAAAATGG TGTAATTGAA CTAGAAGGAAG
5760
CTGCGTACTA TGCCGGCAC ATATTGTACA AAACCGACGA TCCCAAATTC ATTGATTATA
5820
TAAATTAAAT ATTAAAGCA ACACACTCCG AAGAACTACC AGAAAATAGC ACTGTTGTAA
5880
ATTACAGAAA AACTATGCCG AGCGGGTACTA TACACCCCAT TAAAAAAGAC ATATATTTT
5940

FIG. 7J

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ATGACAACAA AAAATTACT CTATACGATA GATACATATA TGGATAACGAT ATAACATATG
TTAATTTTTA TGAGGAGAAA AATGAAAAAG AGAAGGAATA CGAACGAAAGAA GACGCCAAGG
CGTCTAGTTT ATGTGAAAAT AAAATTATAT TGTGCAAAT TAACTGTGAA TCATTGTGAA
ATGATTAA ATATTACCTC AGCGATTATA ACTACGCCGT TTCAATTATA GATAACACTA
CAAATGTTCT TGTTGCCTT GGTTTGATTC GTTAATAAAA AACAAATTAA GCATTATAA
TTGTTTATT ATTCAAATAAT TACAAATAGG ATTGAGACCC TTGCAGTTGC CAGCAAACGG
ACAGAGCTTG TCGAGGGAGAG TTGTTGATTG ATTGTTGCTGC TCCCTGCTGC GTTTTGAC
CGAAGTTCAT GCCAGTCCAG CGTTTTGCA GCAGAAAAGC CGCCGACTTC GGTTTGCGGT
CGCGAGTGAA GATCCCTTTC TTGTTACCGC CAACGCCCAA TATGCCCTGC GAGGTCGCAA
AATCGGGCAA ATTCCATACC TGTTCACCGA CGACGGCGCT GACGCGATCA AAGACGCCGT
GATACATATC CAGCCATGCA CACTGATACT CTTCACTCCA CATGTCGGTG TACATTGAGT
6000 6060 6120 6180 6240 6300 6360 6420 6480 6540 6600

FIG. 7K

GCAGCCCCGC TAACGTATCC ACGCCCGTATT CGGTGATGAT AATCGGGCTGA TGCAGTTCT
6660
CCTGCCAGGC CAGAAGTTCT TTTTCCAGTA CCTTCTTGC CGTTTCCAAA TCGCCGCTTT
6720
GGACATACCA TCCGTAATAA CGGTTCAAGGC ACAGGCACATC AAAGAGATCG CTGATGGTAT
6780
CGGTGTGAGC GTCGCAGAAC ATTACATTGA CGCAGGTGAT CGGACGGCGTC GGGTCCGAGTT
6840
TACGGCGTTGC TTCCGCCAGT GGCGCGAAAT ATTCCCGTGC ACCTTGCGGA CGGGTATCCG
6900
GTTCGTTGGC AATACTCCAC ATCACCACCGC TTGGGTGGTT TTTGTCACGC GCTATCAGCT
6960
CTTAATCGC CTGTAAGTGC GCTTGGTGAG TTTCCCCGTT GACTGGCCTCT TCGTTGTACA
7020
GTTCTTCCG CTTGTTGCC GCTTCAAAAC CAATGCCCTAA AGAGAGGTTA AAGGCCGACAG
7080
CAGCAGTTTC ATCAATCACC ACGATGCCAT GTTCATCTGC CCAGTCGAGC ATCTCTTCAG
7140
CGTAAGGGTA ATGCGAGGTA CGGTAGGGAGT TGGCCCTAAT CCAGTCCATT AATGCGGTGGT
7200
CGTGCACCAT CAGCACCGTTA TCGAATCCCTT TGCCACGCAA GTCCGCATCT TCATGACGAC
7260

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FIG. 7L

CAAAGCCAGT AAAGTAGAAC GGTTTGTGGT TAATCAGGAA CTGTTGCCCTTCACTGCCA
CTGACCGGAT GCCGACGGCA AGCGGGTAGA TATCACACTC TGTCTGGCTT TTGGCTGTGA
7320
CGCACAGTTC ATAGAGATAA CCTTCACCCG GTTGCAGAG GTGCCAGATT ACCACTTGCA
7380
AAGTCCCGCT AGTGCCTTGT CCAGTTGCAA CCACCTGTTG ATCCGCATCA CGCAGTTCAA
7440
CGCTGACATC ACCATTGCC ACCACCTGCC AGTCAACAGA CGCGTGGTTA CAGTCTTGC
7500
CGACATGCGT CACTACGGTG ATATCGTCCA CCCAGGTGTT CGGCCTGGTG TAGAGCATT
7560
CGCTGGATG GATTCCGGCA TAGTTAAAGA AATCATGGAA GTAAGATTGC TTTTCTTGC
7620
CGTTTCTGTT GGTAATCACC ATTCCCCGGCG GGATAGTCTG CCAGTTCAGT TCGTTGTTCA
7680
7740
CACAAACGGT GATACCCCTC GACGGATTAA AGACTTCAG CGGTCAACTA TGAAGAAGTG
7800
TTCGTCTCG TCCCAGTAAG CTATGTCTCT AGAATGTAGC CATCCATCCT TGTCATCAA
7860
GGCGTTGGTC GCTTCCGGAT TGTTTACATA ACCGGACATA ATCATTAGGTC CTCTGACACAA
7920

20/75

FIG. 7M

TAATACGCCT CTCTGATTAA CGCCCCAGCGT TTTCCGGTA TCCAGATCCA AACCTTCGC
7980
TTCAAAAAT GGAACAACTT TACCGACCGC GCCGGTTA TCATCCCCCT CGGGTGTAAAT
8040
CAGAATAGCT GATGTAGTCT CAGTGAGCCC ATATCCTTGT CGTATCCCTG GAAGATGGAA
8100
GCGTTTGCA ACCGCTTCCC CGACTTCTTT CGAAAGAGGT GCGCCCCAG AAGCAAATTTC
8160
GTGTAAATTAA GATAAATCGT ATTTGTCAAT CAGAGTGCCT TTGGCGAAGA ATGAAAATAG
8220
GGTTGGTACT AGCAACGCCAC TTTGAATTAA GTAAATCCTGTA AGGGATCGTA AAAACAGCTC
8280
TTCTTCAAAT CTATACATTA AGACGACTCG AAATCTACAT ATCAAATATC CGAGTGTAGT
8340
AACATTCCA AAACCGTGTGAT GGAATGGAAC AACACTAAA ATCGCAGTAT CGGAATGTAT
8400
TTGATTGCCA AAAATAGGAT CTCTGGCATG CGAGAATCTA GCGCAGGCAG TTCTATGCGG
8460
AAGGGCCACA CCCTTAGGTA ACCCAGTAGA TCCAGAGGAA TTGTTTTGTC ACGATCAAAG
8520
GACTCTGGTA CAAAATCGTA TTCATTAAA CCGGGAGGTA GATGAGATGT GACGGAAGGTG
8580

FIG. 7N

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TACATCGACT GAAATCCCTG GTAATCCGT TTAGAACATT TGATAATAAT TTTCTGGATT
8640
ATTGGTAATT TTTTGCAC GTTCAAAATT TTTGCCAACC CCTTTTGGGA AACAAACACT
8700
ACGGTAGGCT GCGAAATGTT CATACTGTT AGCAATTCACT GTCATTATA AATGTCGTTCT
8760
GGGGCGCAA CTGCAACTCC GATAAATAAC GCCCCAAACA CGGCATAAA GAATTGAAGA
8820
GAGTTTCAC TGCATACGAC GATTCTGTGA TTTGTATTCA GCCCATATCG TTTCATAGCT
8880
TCTGCCAAC GAACGGACAT TTCGAAGTAT TCCGGCTACG TGATGTTCAC CTCGATATGT
8940
GCATCTGTAA AAGGAATTGT TCCAGGAACC AGGGCGTATC TCTTCATAGC CTTATGCAGT
9000
TGCTCTCAG CGGTTCCATT CTCTAGCTTT GCCTCTCAAT TTCTTATTG CATAATGAGA
9060
AAAAAGGAA ATTAATTAA AACACCAATT CAGTAGTTGA TTGAGCAAAT GCCTTGCCTAA
9120
AAAGGATGCT TTAGAGACAG TGTTCCTCTGC ACAGATAAGG ACAAACATCA TTCAGGGGA
9180
GTACCCAGAG CTGAGACTCC TAAGCCAGTG AGTGGCACAG CATTCTAGGG AGAAATATGC
9240

FIG. 7P

TTGTCATCAC CGAAGCCTGA TTCCGTAGAG CCACACCTTG GTAAGGGCCA ATCTGCTCAC
ACAGGATAGA GAGGGCAGGA GCCCAGGGCAG AGCATATAAG GTGAGGTAGG ATCAGTTGCT
CCTCACATT GCTTCTGACA TAGTTGTGTT GGGAGGCTTGG ATCGATCCAC CATGGGCTTC
AATACCCCTGA TTGACTGGAA CAGCTGTAGC CCTGAAACAGC AGCGTGCCT GCTGACGGGT
CCGGCGATT CCGCCTCTGA CAGTATTACC CGGACGGTCA GCGATATTCT GGATAATGCA
AAAACGGCG GTGACGATGC CCTGCGTGA TACAGGGCTA AATTGATAA AACAGAACGTC
ACAGCGCTAC GCGTCACCCC TGAAGAGATC GCCGCCGCCG GCGGCCGTCT GAGCGACGAA
TTAAAACAGG CGATGACCC TCCCCCAA AATATTGAAA CGTTCCATTG CGCCAGGACG
CTACCGCTTG TAGATGTGGA AACCCAGCCA GGCGTGCCTT GCCAGCAGGT TACGGTCCC
GTCTCGTCTG TCGGTCTGTA TATTCCGGC GGCTCGGCTC CGCTCTTCTC AACGGTGTG
ATGCTGGCGA CGCCGGCGC CATTGGGGGA TGCTAGAAGG TGCTTCTGTG CTCGCCGCCG
9300
9360
9420
9480
9540
9600
9660
9720
9780
9840
9900
23/75

FIG. 7Q

24/75

CCCATGGCTG ATGAAATCCT CTATGCCGG CAACTGTGTG GCGTGCAGGA ATTCTTTAAC
CTCGGGGGCG CGCAGGGCAT TGCCGCTCTG GCCTTGGCA GCGAGTCCGT ACCGAAAGTG
9960
GATAAAATT TTGGCCCCGG CAACGCCCTT GTAACCGAAG CCAAACGTCA GGTCAAGCCAG
10020
CGTCTGGACG GCGGGCTAT CGATATGCCA GCCGAGCCGT CTGAAGTACT GGTGATCGCA
10080
GACAGGGCG CAACACCGGA TTTCGTCGCT TCTGACCTGC TCTCCCAGAC TGAGCACGGC
10140
10200
CCGGATTCCC AGGTGATCCT GCTGACGCC GATGCTGACA TTGCCCCGCAA GGTGGCGGAG
10260
GGGGTAGAAC GTCAACTGGC GGAACTGCC CGGGGGACA CGGCCCTGGCA GGCCCTGAGC
10320
GCCAGTCGTC TGATTGTGAC CAAAGATTAA GCGCAGTGC GCG TCGCCATCTC TAATCAGTAT
GGCCGGAAC ACTTAATCAT CCAGACGCC AATGCGCGC ATTTGGTGA TCGGATTACC
10380
10440
AGCGCAGGCT CGGTATTTCT CGGCAGACTGG TCGCCGGAAAT CGGCCGGTGA TTACGCTTCC
10500
GGAACCAACC ATGTTTACCC GACCTATGGC CATACTGCTA CCTGTTCCAG CCTTGGGTTA
10560

FIG. 7R

CCGGATTCC AGAACGGAT GACCGTTTCAG GAACTGTCA G AAGCGGGCTT TTCCGCTCTG
GCATCAACCA TTGAAACATT GGCGGGCA GAACGTTCTGA CCGCCCCATAA AAATGCCGTCG
10620
ACCCTGCAG TAAACGCCCT CAAGGAGCAA GCATGAGCAC TGAAAACACT CTCAGCGTCG
10680
10740
CTGACTTAGC CCGTGAAAT GTCCGCAACC TGAGAGATCCA GACATGATAA GATACATTGA
10800
TGAGTTGGA CAAACCACAA CTAGAATGCA GTGAAAAAAA TGCTTTATT TGAAATTGG
10860
TGATGCTATT GCTTTATTG TAACCATTAT AAGCTGCAAT AAACAAAGTTA ACAACAAACAA
10920
10980
TTGCATTCA TTTATGTTTC AGGTTCAAGGG GGAGGGTGTGG GAGGGTTTTT AAAAGCAAGTA
AACCTCTAC AAATGTGGTA TGGCTGATT TGATCTCTAG CTCGACGGGG CGCCTGGCCG
11040
CTACTAACTC TCTCCTCCCT CCTTTTCCT GCAGGCTCAA GGCGCGCATG CCCGACGGCG
11100
AGGATCTCGT CGTGACCCAT GGCGATGCCT GCTTGCCAA TATCATGGTG GAAAATGGCC
11160
GCTTTCTGG ATTCAATCGAC TGTGGCCGGC TGGGTGTGGC GGACCGCTAT CAGGACATAG
11220

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FIG. 7S

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CGTTGGCTAC CCGTGATATT GCTGAAGAGGC TTGGGGCGA ATGGGCTGAC CGCTTCCTCG
11280
TGCTTTACGG TATCGCCGCT CCCGATTTCGGC AGCGCATTCGC CTTCTATCGC CTTCTTGCACG
11340
AGTTCTTCTG AGCGGGACTC TGGGGTTCGA AATGACCGAC CAAGCGACGC CCAACCTGCC
11400
ATCACGAGAT TTCGATTCCA CGGCCGCCCTT CTATGAAAGG TTGGGCTTCG GAATCGTTT
11460
CGGGGACGCC GGCTGGATGA TCCTCCAGCG CGGGGATCTC ATGCTGGAGT TCTTCGCCCA
11520
CCCCAACTTG TTTATTGCAG CTTATAATGG TTACAAATAA AGCAATAGCA TCACAAATT
11580
CACAAATAA GCATTTTTT CACTGCATTCT TAGTTGTGGT TTGTCCAAAC TCATCAATCT
11640
ATCTTATCAT GTCTGGATCG CGGCCCCGTCT CTCTCTAGGCC CTAGGTCTAG ACTTTGGCAGA
11700
ACATATCCAT CGCGTCCGCC ATCTCCAGCA GCCGCACGCC GCGCATCTCG GGAGGCGTTG
11760
GGTCCTGGCC ACGGGTGCAGC ATGATCGTGC TCCTGTGCTT GAGGACCCGG CTAGGCTGGC
11820
GGGGTTGCCCT TACTGGTTAG CAGAATGAAT CACCGATAACG CGAGCGAACG TGAAGCGACT
11880

FIG. 7T

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GCTGCTGCAA AACGTCTGCG ACCTGAGCAA CAAACATGAAT GGTCTTCGGT TTCCGGTT
11940
CGTAAAGTCT GGAAACGGGG AAGTCAGGCC CCTGCACCAT TATGTTCCGG ATCTGCATCG
12000
CAGGATGCTG CTGGCTACCC TGTGGAACAC CTACATCTGT ATTAACGAAG CGCTGGCATT
12060
GACCCTGAGT GATTTTCTC TGGTCCGCC GCATCCATAAC CGCCAGTTGT TTACCCCTCAC
12120
AACGTTCCAG TAACCGGGCA TGTTCATCAT CAGTAACCCG TATCGTGAGC ATCCCTCTC
12180
GTTTCATCGG TATCATTACC CCCATGAACA GAAATCCCCC TTACACGGAG GCATCAGTGA
12240
CCAACAGGA AAAAACGCC CTTAACATGG CCCGCTTAT CAGAACGCCAG ACATTAACGC
12300
TTCTGGAGAA ACTCAACGAG CTGGACGGCG ATGAACAGGG AGACATCTGT GAATCCCTC
12360
ACGACCACGC TGATGAGCTT TACCGCAGCT GCCTCGCGCG TTTGGTGAT GACGGTGAAA
12420
ACCTCTGACA CATGCAGCTC CCGGAGACGG TCACAGCTG TCTGTAAGCG GATGCCGGGA
12480
GCAGACAAGC CCGTCAGGGC GCGTCAGCGG GTGTTGGCGG GCAGGCCATGA
12540

FIG. 7U

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CCCAGTCACG TAGCGATAGC GGAGGTGTATA CTGGCTTAAC TATGCCGCAT CAGAGCAGAT
12600
TGTACTGAGA GTGCACCAT A TGCGGTGTGA AATACCGCAC AGATGCGTAA GGAGAAAATA
12660
CCGCATCAGG CGCTCTCCG CTTCCCTCGCT CACTGACTCG CTGGCGCTCGG TCGGTTCGGCT
12720
GGGGCAGCG GTATCAGCTC ACTCAAAGGC GGTAAATACGG TTATCCACAG AATCAGGGGA
12780
TAACGCAGGA AAGAACATGT GAGCAAAAGG CCAGCAAAAG GCCAGGAACC GTAAAAAGGC
12840
CGCGTTGCTG GCGTTTTCC ATAGGCTCCG CCCCCCTGAC GAGGCATCACA AAAATCGACG
12900
CTCAAGTCAG AGGTGGGAA ACCCGACAGG ACTATAAAGA TACCAAGGGT TTCCCCCTGG
12960
AAGCTCCCTC GTGCGCTCTC CTGTTCCGAC CCTGGCCGCTT ACCGGATACC TGTCCGCCCT
13020
TCTCCCTCG GGAAAGCGTGG CGCTTTCTCA TAGCTCACGC TGTAGGTATC TCAGTTGGT
13080
GTAGGTCGTT CGCTCCAAGC TGGGCTGTGT GCACGAACCC CCCGTTCAAGC CCGACCGCTG
13140
CGCCTTATCC GGTAACTATC GTCTTGAGTC CAACCCGGTA AGACACGACT TATCGCCACT
13200

FIG. 7V

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GGCAGCGGCC ACTGGTAACA GGATTAGCAG AGCGGAGGTAT GTAGGGGGTG CTACAGAGTT
CTTGAAGTGG TGGCCTAACT ACGGCCTACAC TAGAAGGACA GTATTGGTA TCTGCCGCTCT
GCTGAAGCCA GTTACCTTCG GAAAAAGAGT TGGTAGCTCT TGATCCGGCA AACAAACCAC
CGCTGGTAGC GGTGGTTTTT TTGTTTGCAA GCAGCAGATT ACGCCGCAGAA AAAAGGATC
TCAAGAAGAT CCTTGATCT TTTCTACGGG GTCTGACGCT CAGTGGAACG AAAACTCACG
TTAAGGGATT TTGGTCATGA GATTATCAA AAGGATCTTC ACCTAGATCC TTTTAAATTA
AAAATGAAGT TTAAATCAA TCTAAAGTAT ATATGAGTAA ACTTGGTCTG ACAGTTACCA
ATGCTTAATC AGTGAGGCAC CTATCTCAGC GATCTGTCTA TTTCGTTCAT CCATAGTTGC
CTGACTCCCC GTCGTGTAGA TAACTACGAT ACGGGAGGGC TTACCATCTG GCCCCAGTGC
TGCAATGATA CCGCGAGACC CACGCTCACC GGCTCCAGAT TTATCAGCAA TAAACCAGCC
AGCCGGAAAGG GCCGAGGCC GAAGTGGTCC TGCAACTTTA TCCGGCTCCA TCCAGTCTAT
13260
13320
13380
13440
13500
13560
13620
13680
13740
13800
13860

FIG. 7W

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TAATTGTGTC CGGGAAAGCTA GAGTAAGTAG TTCGCCAGTT AATAGTTTGC GCAACGTTGT
 13920
 TGCATGGCT GCAGGCATCG TGGTGTCAAG CTCGGTGGTT GGTATGGCTT CATTCAAGCTC
 13980
 CGGTTCCCAA CGATCAAGGC GAGTTACATG ATCCCCATG TTG TGCAAAA AAGCGGGTTAG
 14040
 CCTCTCGGT CCTCCGATCG TTGTCAGAAG TAAGTTGCC GCAGTGTAT CACTCATGGT
 14100
 TATGGCAGCA CTGCATAATT CTCTTACTGT CATGCCATCC GTAAGATGCT TTTCTGTGAC
 14160
 TGGTGAGTAC TCAACCAAGT CATTCTGAGA ATAGTGTATG CGGCCGACCGA GTTGCTCTTG
 14220
 CCCGGCGTCA ACACGGATA ATACCGCGCC ACATAGCAGA ACTTTAAAG TGCTCATCAT
 14280
 TGGAACACGT TCTTCGGGC GAAAACCTCTC AAGGATCTTA CCGCTGTGTA GATCCAGTTTC
 14340
 GATGTAACCC ACTCGTGCAC CCAAATGATC TTCAAGCATCT TTTACTTTCA CCAGCGTTTC
 14400
 TGGGTGAGCA AAAACAGGAA GGCAAAATGC CGCAAAAGGG GGAATAAGGG CGACACGGAA
 14460
 ATGTTGAATA CTCATACTCT CCCTTTTCA ATATTGA AGCATTATC AGGGTTATTG
 14520

FIG. 7X

TCTCATGAGC GGATACATAT TTGAATGTAT TTAGAAAAAT AAACAAATAG GGGTTCCGCG
14580
CACATTCCC CGAAAAGTGC CACCTGACGT CTAAGAAACC ATTATTATCA TGACATTAAAC
14640
CTATAAAAT AGGCGTATCA CGAGGCCCTT TCGTCTTCAA GAA
14683

FIG. 8A

TTAATTAAGG GGCGGGAAAT GGCGGGAACT GGGCGGGACTT AGGGGGGGGA TGGGGGGAGGT 60
 TAGGGGGGG ACTATGGTTG CTGACTAATT GAGATGCATG CTTTGCATAC TTCTGCCCTGC 120
 TGGGGAGCCT GGGGACTTTTC CACACCTGGT TGCTGACTAA TTGAGATGCA TGCTTTGCAT
 ACTTCTGCCT GCTGGGGAGC CTGGGGACTT TCCACACCCCT AACTGACACA CATTCACAG 180
 ATTAAATTCC CCTAGTTTATT AATAAGTAATC ATTACGGGG TCATTAGGTC ATAGCCCCATA 240
 TATGGAGTTTC CGCGTTACAT AACCTTACGGT AAATGGCCCG CCTGGCTGAC GCCAACGAA 300
 CCCCCGCCA TTGACCGTCAA TAATGACGTA TGTTCCCATA GTAACGCCAA TAGGGACTTT 360
 CCATTGACGT CAATGGGTGG ACTATTACG GTAAACTGCCC CACCTGGCAG TACATCAAGT 420
 GTATCATATG CCAAGTACGC CCCCTATTGA CGTCAATGAC GGTAATGGC CGCCTGGCA 480
 TTATGCCAG TACATGACCT TATGGGACTT CCCTACTTGG CAGTACATCT ACGTATTAGT 540
 CATCGCTATT ACCATGGTGA TGCGGTTTG GCAGTACATC AATGGGGGTG GATAGGGTT 600
 TGACTCACCGG GGATTCCCAA GTCTCCACCC CATTGACGTC AATGGGAGTT TGTTTGAAAG
 CTTGGCCGGC CATATAAACG GCGGCCAGCT TTATTAAACG TGTTTACGTC GAGTCAAATTG 660
 TACACTAACG ACAGTGATGA AAGAAATACA AAAGGCCATA ATATTGGAA CGACGTCGAA 720
 32/75

FIG. 8B

CCTTTATTAC AAAACAAAC ACAAACGAAT ATCGACAAAG CTAGGATTGCT GCTACAAGAT 900
 TTGGCAAGTT TTGTGGCGTT GAGCGAAAAT CCATTAGATA GTCCAGCCAT CGGTTCGGAA 960
 AAACAACCT TGTGAAAC TAATCGAAAC CTATTTCACA AATCTATTGA GGATTAAATA 1020
 TTTAAATTCA GATAAAAGA CGCTGAAAAT CATTGATT TCGCTCTAAC ATACCACCCCT 1080
 AAAGATTATA ATTAAATGA ATTATTAAGA TACATCAGCA ACTATATATT GATAGACATT 1140
 TCCAGTTTGT GATATTAGTT TGTGCGTCTC ATTACAATGG CTGTTATTCT TAACAAACAA 1200
 CAACTGCTCG CAGACAATAG TATAGAAAAG GGAGGGTGAAAC TGTTTGTGTT TAACGGTTCG 1260
 TACAACATT TGGAAAGTTA TGTTAATCCG GTGCTGCTAA AAAATGGTGT ATTGAACTA 1320
 GAAGAGCTG CGTACTATGC CGGCAACATA TTGTACAAA CCGACGATCC CAAATTCACT 1380
 GATTATATA ATTAAATAAT TAAAGCAACA CACTCCGAAG AACTACCAGA AAATAGCACT 1440
 GTTGTAATT ACAGAAAAC TATGCGCAGC GGTACTATAC ACCCCATTAA AAAAGACATA 1500
 TATTTTATG ACAACAAAAA ATTTACTCTA TACGATAGAT ACATATATGG ATACGATAAT 1560
 AACATTGTTA ATTITATGA GGAGAAAAT GAAAAGAGA AGGAATAACGA AGAAGAAGAC 1620
 GACAAGGGCGT CTAGTTTATG TGAAAATAAA ATTATATTGT CGCAAATTAA CTGTGAATCA 1680

33/75

FIG. 8C

TTGAAATG ATTTAAATA TTACCTCAGC GATTAAACT ACGCGTTTC AATTATAGAT 1740
AATACTACAA ATGTTCTTGT TGCCTGGT TTGTATCGTT AAAAAAAC AAATTTAGCA 1800
TTTATAATTG TTTTATTATT CAATAATTAC AAATAGGATT GAGACCCTTG CAGTGGCCAG 1860
CAAACGGACA GAGCTTGTAG AGGAGAGTTG TTGATTCAATT GTTGCCTTCC CTGCTGCCGT 1920
TTTCACCGA AGTTCATGCC AGTCCAGCGT TTTTGCAGCA GAAAAGCCGC CGACTTCGGT 1980
TTGCGGTCGC GAGTGAAGAT CCCTTCTTG TTACGCCAA CGCGCAATAT GCCTTGCGAG 2040
GTGGCAAAT CGCCGAAATT CCATACCTGT TCACCGACGA CGGGGCTGAC GCGATCAAAG 2100
ACGGGGTGTACATACATCCAG CCATGCACAC TGATACTCTT CACTCCACAT GTCGGGTGTAC 2160
ATTGAGTGCA GCCCGGCTAA CGTATCCACG CCGTATTCTGG TGATGATAAT CGGCTGATGC 2220
AGTTTCTCCT GCCAGGCCAG AAGTTCTTT TCCAGTACCT TCTCTGCCGT TTCCAAATCG 2280
CCGCTTTGGA CATAACCATT GTAATAACGG TTCAGGGCACA GCACATCAA GAGATCGCTG 2340
ATGGTATCGG TGTGAGCGTC GCAGAACATT ACATTGACGC AGGTGATCGC ACGCGTCCGG 2400
TCGAGTTAC GCGTTGCTTC CGCCAGTGGC GCGAAATATT CCCGTGCCACC TTGGGGACGG 2460
GTATCCGGTT CGTTGGCAAT ACTCCACATC ACCACGCTTG GGTGGTTTT GTCACGGCCT 2520
34/75

FIG. 8D

ATCAGCTCTT TAATGGCCCTG TAAGGTGCCGT TGCTGAGGCT CCCCGTTGAC TGCCTCTTCG 2580.
 CTGTACAGTT CTTTCGGCTT GTGAAACCAA TCGAAACCAA TGCCCTAAAGA GAGGTTAAAG 2640
 CCGACAGCAG CAGTTTCATC AATCACCACTG ATGCCATGTT CATCTGCCA GTCGAGGCATC 2700
 TCTTCAGCGT AAGGGTAATG CGAGGGTACGG TAGGAGTTGG CCCCAATCCA GTCCATTAAAT 2760
 GCGTGGTCGT GCACCATCAG CACGTTATCG AATCCCTTGC CACGCAAGTC CGCATCTTC 2820
 TGACGACCAA AGCCAGTAAA GTAGAACCGGT TTGTGGTTAA TCAGGAACTG TTGCCCTTC 2880
 ACTGCCACTG ACCGGATGCC GACGGGAAGC GGGTAGATAT CACACTCTGT CTGGCTTTTG 2940
 GCTGTGACGC ACAGTTCATA GAGATAACCT TCACCCGGTT GCCAGAGGTG CGGATTCAACC 3000
 ACTTGCAAAG TCCCCTAGT GCCCTGTCCA GTTGCAACCA CCTGTTGATC CGCATCACGC 3060
 AGTTCAACGC TGACATCACC ATTGGCCACC ACCTGCCAGT CAACAGACGC GTGGTTACAG 3120
 TCTTGGCGA CATGCCGTAC CACGGTATA TCGTCCACCC AGGTGTTGG CCTGGTTGTAG 3180
 AGCATTACGC TCGGATGGAT TCCGGCATAG TTAAAGAAAT CATGGAAGTA AGACTGCTTT 3240
 TTCTTGGCGT TTTCGGTGGT AATCACCAATT CCCGGGGGA TAGTCTGCCA GTTCAGTTTCG 3300
 TTGTTCACAC AACCGGGTGTAC ACCCCTCGAC GGATTAAAGA CTTCAAGGGG TCAACTATGA 3360

35/75

FIG. 8E

AGAAGTGTTC GTCTTCGTCC CAGTAAGCTA TGTCTCCAGA ATGTAGCCAT CCATCCTTGT 3420
CAATCAAGGC GTGGGTCGCT TCCGGATTGT TTACATAACC GGACATAATC ATAGGTCCCTC 3480
TGACACATAA TTCGCCCTCTC TGATTAACGC CGAGCGTTT CCCGGTATCC AGATCCACAA 3540
CCTTCGGCTTC AAAAAATGGA ACAACTTAC CGAACCGGCC CGGTTTATCA TCCCCCTCGG 3600
GTGTAATCAG AATAGCTGTAT GTAGTCTCAG TGAGCCCATA TCCTTGTCTGTT 3660
GATGGAAGGCG TTTTGCAACC GCTTCCCGA CTTCTCGA AAGAGGTGCG CCCCGAGAAG 3720
CAATTTCGGT TAAATTAGAT AAATCGTATT TGTCAATCAG AGTGCCTTTG GCGAAGAATG 3780
AAAATGGGT TGGTACTAGC AACGCCACTTT GAATTGGTAA ATCCTGAAGG GATCGTAAAA 3840
ACAGCTCTTC TTCAAATCTA TACATTAAGA CGACTCGAAA TCCACATATC AAATATCCGA 3900
GTGTAGTAAA CATTCCAAA CCGTGATGGA ATGGAACAAC ACTTAAAATC GCAGTATCCG 3960
GAATGATTG ATTGCCAAA ATGGATCTC TGGCATGCGA GAATCTAGCG CAGGCAGTTC 4020
TATGCGGAAG GCCCACACCC TTAGGTAACC CAGTAGATCC AGAGGAATTG TTTTGTCAAG 4080
ATCAAAGGAC TCTGGTACAA AATCGTATTG ATAAAACCG GGAGGTAGAT GAGATGTCAC 4140
GAACGTGTAC ATCGACTGAA ATCCCTGGTA ATCCGTTTA GAATCCATGA TAATAATT 4200

36/75

FIG. 8F

CTGGATTATT GGTAATTTT TTTGCACCGTT CAAAATTTTG TGCAAACCCCT TTTTGGAAAC 4260
AACACTACG GTAGGGCTGCG AAATGTTCAT ACTGTTGAGC AATTCACTGTT CATTATAAAT 4320
GTCGTTGGCG GGCGCAACTG CAACTCCGAT AAATAACGCG CCCAACACCCG GCATAAAGAA 4380
TTGAAGAGAG TTTTCACTGCA ATACGACCGAT TCTGTGATT GTATTCAAGCC CATATCGTTT 4440
CATAGCTTCT GCCAACCGAA CGGACATTTC GAAGTATTCC GCGTACGTGA TGTTCACCTC 4500
GATATGTC GCA TCTGTAAAAG GAATTGTTCC AGGAACCAGG GCGTATCTCT TCATAGCCTT 4560
ATGCAGTTGC TCTCCAGCGG TTCCATCCCTC TAGCTTTGCT TCTCAATTTC TTATTTGCAT 4620
AATGAGAAAA AAAGGAAAAT TAATTTAAC ACCAATTTCAG TAGTTGATTG AGCAAATGCG 4680
TTGCCAAAA GGATGCTTA GAGACAGTGT TCTCTGCACA GATAAGGACA AACATTATTC 4740
AGAGGGAGTA CCCAGAGCTG AGACTCCCAA GCCAGTGAGT GGCACAGCAT TCTAGGGAGA 4800
AATATGCTTG TCATCACCGA AGCCTGATTG CGTAGAGCCA CACCTTGGTA AGGGCCAATC 4860
TGCTCACACA GGATAGAGAG GGCAGGGGCC AGGGCAGAGC ATATAAGGTG AGGTAGGGATC 4920
AGTTGCTCCT CACATTGCT TCTGACATAG TTGTGTTGGG AGCTTGGATC GATCCACCAT 4980
GGGCTTCAAT ACCCCTGATTG ACTGGAACAG CTGTAGCCCT GAACAGCAGC GTGCGCTGCT 5040

FIG. 8G

40/41040
PCT/US98/03935

GACGGCTCCG GCGATTTCGG CCTCTGACAG TATTACCCGG ACGGTCAAGC 5100
TAATGTAAAA ACGCGGGTG ACGATGCCCT GCGTGAATAC AGCGCTAAAT TTGATAAAC 5160
AGAAGTGACA GCGCTACGGC TCACCCCTGA AGAGATCGCC GCCGCCGGCG CGCGTCTGAG 5220
CGACGAATTA AACAGGGGA TGACCGCTGC CGTCAAAAT ATTGAAACGT TCCATTCCGC 5280
GCAGACGCTA CCGCCTGTAG ATGTGGAAAC CCAGCCAGGC GTGCGTTGCC AGCAGGTTAC 5340
GGGTCCCGTC TCGTCTGTGC GTCTGTATAT TCCCGGGGCC TCGGCTCCGC TCTTCTCAAC 5400
GGTGCTGATG CTGGCGACGC CGGGGGCAT TGCGGGATGC CAGAAGGTGG TTCTGTGCTC 5460
GCCGCCGCC ATCGCTGATG AAATCCTCTA TGCGGGCAA CTGTGTGCC TGAGGAAAT 5520
CTTAAACGTC GGCGGGCGC AGGGGATTGC CGCTCTGGCC TTGGCAGGCC AGTCCGTACC 5580
GAAAGTGGAT AAAATTNTTG GCCCGGGCAA CGCCTTGTAA ACCGAAGCCA AACGTCAGGT 5640
CAGCCAGCGT CTCGACGGCG CGGCTATCGA TATGCCAGGC GGGCGTCTG AAGTACTGGT 5700
GATCGCAGAC AGCGGGCAA CACCGGATT CGTCGCTTCT GACTGCTCT CCCAGGCTGA 5760
GCACGGCCG GATTCCCAGG TGATCCTGCT GACGGCTGAT GCTGACATTG CCCGCAAGGT 5820
GGCGGAGGGCG GTAGAACGTC AACTGGGGAA ACTGCCGGC GCAGCACCCG CCCGGCAGGC 5880

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FIG. 8H

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PC17US98/03935

CCTGAGGCC AGTCGGTCTGA TTGTGACCAA AGATTAGCG CAGTGCCTCG CCATCTCTAA 5940
TCAGTATGGG CGGGAACACT TAATCATCCA GACGGCCAAAT GCGCGCGATT TGGTGGATGC 6000
GATTACCAGC GCAGGGCTCGG TATTTCCTCGG CGACTGGTGG CCGGAATCCG CCGGTGATTAA 6060
CGCTTCCGA ACCAACCCATG TTTTACCGAC CTATGGCTAT ACTGCTACCT GTTCCAGGCCT 6120
TGGGTTAGG GATTTCCAGA AACGGATGAC CGTTCAAGGAA CTGTCGAAAG CGGGCTTTTC 6180
CGCTCTGGCA TCAACCCATTG AAACATTGGC GGCGGCCAGAA CGTCTGACCC CCCATAAAA 6240
TGCCGTGACC CTGGCGGTAA ACCGGCCTCAA GGAGCAAGCA TGAGGCACTGA AAACACTCTC 6300
AGCGTCGCTG ACTTAGCCCCG TGAAAATGTC CGCAACCTGG AGATCCAGAC ATGATAAGAT 6360
ACATTGATGA GTTGGACAA ACCACAACTA GAATGCAGTG AAAAAATGC TTTATTGTG 6420
AAATTTGTGA TGCTATTGCT TTATTGTAA CCATTATAAG CTGCAATAAA CAAGTTAACAA 6480
ACAACAATTG CATTCAATTG ATGTTTCAGG TTCAAGGGGA GGTGTGGAG GTTTTTAA 6540
GCAAGTAAA CCTCTACAAA TGTGGTATGG CTGATTATGA TCTCTAGCTC GACGGGGCGC 6600
CTCTAGAGCA GTGTTGGTTT GCAAGAGGAA GCAAAAAAGCC TCTCCACCA GGCCTGGAAT 6660
GTTCCACCC AATGTCGAGC AGTGTGGTTT TGCAAGAGGA AGCAAAAGC CTCTCCACCC 6720

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FIG. 8I

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AGGCCTGGAA TGTTCCACC CAATGTCGAG CAAACCCGC CCAGCGTCTT GTCATTGGCG 6780
AATCGAACCA CGCAGATGCA GTCGGGCGG CGGGTCCCA GTCCCACCTTC GCATATTAAG 6840
GTGACGGTG TGGCTCGAA CACCGAGCGA CCCTGCAGGCC AATATGGAT CGGCCATTGA 6900
ACAAGATGGA TTGCACCGCAG GTTCTCCGGC CGCTTGGGTG GAGAGGCTAT TCGGCTATGA 6960
CTGGCACAA CAGACAATCG GCTGCTCTGA TGCCGCCGTG TTCCGGCTGT CAGCGCAGGG 7020
GCGCCCGT CTTTTGTCA AGACCGACCT GTCCGGTGCC CTGAATGAAC TGCAGGTAAG 7080
TGCGGCCGTC GATGGCCGAG GCGGCCTCGG CCTCTGCATA AATAAAAAAA ATTAGTCAGC 7140
CATGCATGGG GCGGAGGAATG GGCGGAACTG GGCGGAGTTT GGGCGGGAT GGGGGAGTT 7200
AGGGGGGA CTATGGTTGC TGACTAATTG AGATGCATGC TTTGCATACT TCTGCCTGCT 7260
GGGGAGCCTG GGGGACTTTCC ACACCTGGTT GCTGACTAAT TGAGATGCAT GCTTTGCATA 7320
CTTCTGCCTG CTGGGGAGCC TGGGACTTT CCACACCCCTA ACTGACACAC ATTCCACAGA 7380
ATTAATTCCC CTAGTTATTA ATAGTAATCA ATTACGGGGT CATTAGTTCA TAGCCCCAT 7440
ATGGAGTTCC GCGTTACATA ACTTACGGTA ATGGGGGGC CTGGCTGACC GCCCAAACGAC 7500
CCCCGCCAT TGACGTCAAT AATGACGTAT GTTCCCCATAG TAACGCCAAT AGGGGACTTTTC 7560

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FIG. 8J

CATTGACCGTC AATGGGGTGGG CTATTTACGG TAAACTGCC ACTTGGCAGT ACATCAAAGTG 7620
TATCATATGC CAAGTACGCC CCCTATTGAC GTCAATGACG GTAAATGGCC CGCCTGGCAT 7680
TATGCCAGT ACATGACCTT ATGGGACTTT CCTACTTGGC AGTACATCTA GCTATTAGTC 7740
ATCGCTATTAA CCATGGGTGAT GCGGTTGG CAGTACATCA ATGGGCGTGG ATAGCGGGTT 7800
GAECTCACGGG GATTTCCAAG TCTCCACCCC ATTGACGTCG ATGGGAGTTT GTTTGGCAC 7860
CAAATCAAC GGGACTTTCC AAAATGTCGT AACAACTCCG CCCCATGAC GCAAATGGGC 7920
GGTAGGGCGTG TACGGGTGGG AGCAGAGCTG GGTACG TGAA CCGTCAGATC 7980
GCCTGGAGAC GCCATCACAG ATCTCTCACT ATGGGATTTC AGGTGCAGAT TATCAGCTTC 8040
CTGCTAATCA GTGCTTCAGT CATAATGTCC AGAGGACAAA TTGGTTCTC CCAGTCTCCA 8100
GCAATCCCTGT CTGCATCTCC AGGGGAGAAG GTCACAAATGA CTTGCAGGGC CAGCTCAAGT 8160
GTAAGTTACA TCCACTGGTT CCAGGCAGAAG CCAGGATCCT CCCCAAAACC CTGGATTAT 8220
GCCACATCCA ACCTGGCTTC TGGAGTCCT GTTCGCTTCA GTGGCAGTGG GTCTGGGACT 8280
TCTTACTCTC TCACAATCAG CAGAGTGGAG GCTGAAAGATG CTGCCACTTA TTACTGCCAG 8340
CAGTGGACTA GTAACCCACC CACGTTGGGA GGGGGACCA AGCTGGAAAT CAAACGGTACG 8400

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FIG. 8K

GTGGCTGCAC CATCTGTCTT CACCTCCCCG CCATCTGATG AGCAGTTGAA ATCTGGAACT 8460
GCCCTCTGTTG TGTGCTGT GAATAACTTC TATCCAGAG AGGCCAAGT ACAGTGGAAAG 8520
GTGGATAACG CCCTCCAATC GGGTAACTCC CAGGAGAGTG TCACAGAGCA GGACAGCAAG 8580
GACAGCACCT ACAGCCTCAG CAGCACCCCTG ACGCTGAGCA AAGCAGACTA CGAGAAACAC 8640
AAAGTCTACG CCTGCGAAGT CACCCATCAG GGCCTGAGCT CGCCCGTCAC AAAGAGCTTC 8700
AACAGGGAG AGTGGTGAAT TCAGATCCGT TAACGGTTAC CAACTACCTA GACTGGATTTC 8760
GTGACAACAT GCGGGCCGTGA TATCTACGTA TGATCAGGCCT CGACTGTGCC TTCTAGTTGC 8820
CAGCCATCTG TTGTTGCC CTCCCCGGTGA CCTTCCCTTGA CCCTGGAAAGG TGCCACTCCC 8880
ACTGTCCCTT CCTAATAAAA TGAGGAAATT GCATCGCATT GTCTGAGTAG GTGTCAATTCT 8940
ATTCTGGGG GTGGGGTGGG GCAGGACAGC AAGGGGAGG ATTGGGAGA CAATAGCAGG 9000
CATGCTGGGG ATGCCGTGGG CTCTATGGAA CCAGCTGGG CTCGACAGCT ATGCCAAGTA 9060
CGCCCCCTAT TGACGTCAAAT GACGGTAAAT GCCCTGGCCTG GCATTATGCC CAGTACATGA 9120
CCTTATGGGA CTTTCTACT TGCAGTACA TCTACGTATT AGTCATCGCT ATTACCATGG 9180
TGATGCGGTT TTGGCAGTAC ATCAAATGGGC GTGGATAGCC GTTTGACTCA CGGGGATTTC 9240

42/75

FIG. 8L

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CAAGTCTCCA CCCCATTGAC GTCAATGGGA GTTTGTTTG GCACCAAAAT CAACGGGACT 9300
TTCCAAATG TCGTAACAAC TCCGCCCAT TGACGCAAAT GGGCGGTAGG CGTGTACGGT 9360
GGGAGGTCTA TATAAGCAGA GCTGGGTACG TCCTCACATT CAGTGATCAG CACTGAACAC 9420
AGACCCGTCG ACATGGGTTG GAGCCTCATC TTGCTCTTCC TTGTCGCTGT TGCTACGGGT 9480
GTCCTGTCCC AGGTACAACG GCAGGCAGCCT GGGGCTGAGC TGGTGAAGGCC TGGGGCCTCA 9540
GTGAAGATGT CCTGCAAGGC TTCTGGCTAC ACATTTACCA GTTACAATAT GCACTGGTA 9600
AACAGACAC CTGGTGGGG CCTGGAAATGG ATTGGAGGCTA TTTATCCGG AAATGGTGAT 9660
ACTTCCTACA ATCAGAAGTT CAAAGGCAAG GCCCACATTGA CTGCAGACAA ATCCTCCAGC 9720
ACAGCCTACA TGCAGCTCAG CAGCCTGACA TCTGAGGGACT CTGCGGTCTA TTACTGTGCA 9780
AGATCGACTT ACTACGGGG TGACTGGTAC TTCAATGTCT GGGGGCAGG GACCACGGTC 9840
ACCGTCTG CAGCTAGCAC CAAGGGCCA TCGGTCTTCC CCCTGGCACCC CTCCTCCAAG 9900
AGCACCTCTG GGGCACAGC GGCCCTGGGC TGCGTGGTCA AGGACTACTT CCCCCGAACCG 9960
GTGACGGTGT CGTGGAACTC AGGGGCCCTG ACCAGGGCCCTG TGCACACCTT CCGGGCTGTC 10020
CTACAGTCCT CAGGACTCTA CTCCCCTCAGC AGCGTGGTGA CCGTGCCTC CAGCAGCTTG 10080

FIG. 8M

GGCACCCAGA CCTACATCTG CAACGTGAAT CACAAGCCAA GGTGGACAAG 10140
AAAGCAGAGC CCAAATCTTG TGACAAACTCACACATGCC CACCGTGCC AGCACCTGAA 10200
CTCCTGGGG GACCGTCAGT CTTCCCTCTTC CCCCAAAAC CCAAGGACAC CCTCATGATC 10260
TCCGGACCC CTGAGGTAC ACAGCACGTA CGGTGTGGTC AGCGTCCTCA CCAGGACTGG 44/75
AAGTTCAACT GGTACGGTGA CGGGCGTGGAG GTGCATAATG CCAAGACAAA GCCGGGGAG 10320
GAGCAGTACA AGCTGGATG AGCTGGTACAC AGGTGTACAC CCTGCCCA 10380
CTGAATGGCA AGGAGTACAA GTGCAAGGTC TCCAACAAAG CCCTCCCAGC CCCCATCGAG 10440
AAACCATCT CCAAAGCCAA AGGGCAGCCC CGAGAACACC AGGTGTACAC CCTGCCCA 10500
TCCGGGGATG AGCTGACCA GAACCAGGTC AGCCTGACCT GCCTGGTCAA AGGCTTCTAT 10560
CCCAGGGACA TGGCGTGGA GTGGAGGAGC AATGGGGAGC CGGAGAACAA CTACAAGACC 10620
ACGGCTCCG TGCTGGACTC CGACGGCTCC TTCTTCCTCT ACAGCAAGCT CACCGTGGAC 10680
AAGAGCAGGT GGCAGCAGGG GAAACGTCTTC TCATGCTCCG TGATGCATGA GGCTCTGCAC 10740
AACCACTACA CGCAGAAGAG CCTCTCCCTG TCTCCCCCTG AATGAGGATC CGTTAACGGT 10800
TACCAACTAC CTAGACTGGA TTCCGTGACAA CATGCGGGCCG TGATATCTAC GTATGATCAG 10860
TACCAACTAC CTAGACTGGA TTCCGTGACAA CATGCGGGCCG TGATATCTAC GTATGATCAG 10920

FIG. 8N

CCTCGACTGT GCCTTCTAGT TGCCAGCCAT CTGTTGTTG CCCACTGCC
 TGACCCCTGGA AGGTGCCACT CCCACTGTCC TTTCCTAATA AAATGAGGAA ATTGCATCGC 10980
 ATTGCTGAG TAGGTGTCAT TCTATTCTGG GGGGTGGGT GGGCAGGAC AGCAAGGGGG 11040
 AGGATTGGGA AGACAAATAGC AGGCATGCTG GGGATGCGGT GGGCTCTATG GAACCAGGCTG 11100
 GGGCTCGACA GCAACGCTAG GTCGAGGCCG CTACTAACTC TCTCCTCCCT CCTTTCCCT 11160
 GCAGGACGAG GCAGCGGGC TATCGTGCTT GGCCACGACG GGCCTTCCCTT GGCAGCTGT 11220
 GCTCGACGTT GTCACTGAAG CGGAAAGGGAA CTGGCTGCTA TTGGCGGAAG TGCCGGGCA 11280
 GGATCTCCTG TCATCTCACC TTGCTCCTGC CGAGAAAGTA TCCATCATGG CTGATGCAAT 11340
 CGGGCGCTG CATACTGCTTG ATCCGGCTAC CTGCCATTG GACCACCAAG CGAACATATCG 11400
 CATCGAGCGA GCACGTTACTC GGATGGAAGC CGGTCTTGTG GATCAGGATG ATCTGGACGA 11460
 AGAGCATCAG GGGCTCGCGC CAGCCGAACG GTTCGCCAGG TAAGTGAGCT CCAATTCAAG 11520
 CTTCCCTAGGG CGGCCAGGCTA GTAGCTTGC TTCTCAATT CTTATTGCA TAATGAGAAA 11580
 AAAAGGAAA TTAATTAA CACCAATTCA GTAGTTGATT GAGCAAATGC GTTGCCAAA 11640
 AGGATGCTTT AGAGACAGTG TTCTCTGCAC AGATAAGGAC AACACATTATT CAGAGGGAGT 11700
 AGGATGCTTT AGAGACAGTG TTCTCTGCAC AGATAAGGAC AACACATTATT CAGAGGGAGT 11760

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FIG. 8P

ACCCAGAGCT GAGACTCCTA AGGCCAGTGGAG, TGGCACAGCA TTCTAGGGAG AAATATGCTT
GTCATCACCG AAGCCTGATT CCGTAGAGGC ACACCTTGGT AAGGGCCAAT CTGCTCACAC 11880
AGGATAGAGA GGGCAGGAGC CAGGGCAGAG CATATAAGGT GAGGTAGGAT CAGTTGCTCC 11940
TCACATTGC TTCTGACATA GTTGTGTTGG GAGCTTGGAT AGCTTGGACA GCTCAGGGCT 12000
GGGATTTCGC GCCAAACTTG ACGGCAATCC TAGCGGTGAAG GCTGGTAGGA TTTTATCCCC 12060
GCTGCCATCA TGGTTCGACC ATTGAAC TGTC ATCGTCGCC TGTCCTCGG CTCAGGAACG AGTTCAAGTA CTTCCAAAGA 12120
GGCAAGAACG GAGACCTACC CTGGCCTCCG AGTTCTCAGT GGAAAGGTAAA CAGAATCTGG TGATTATGG TAGGAAACC 12180
ATGACCAAA CCTCTTCAGT GGAAAGGTAAA CAGAATCTGG TGATTATGG TAGGAAACC 12240
TGGTTCTCCA TTCCCTGAGAA GAATCGACCT TTAAAGGACA GAATTAAATAT AGTTCTCAGT 12300
AGAGAACTCA AAGAACCC ACCGGGAGCT CATTTCCTTG CCAAAAGTTT GGATGATGCC 12360
TTAAGACCTTA TTGAACAAACC GGAAATTGGCA AGTAAAGTAG ACATGGTTG GATACTGGGA 12420
GGCAGTTCTG TTTACCGGA AGCCATGAAT CAACCAAGGCC ACCCTTAGACT CTTTGTGACA 12480
AGGATCATGC AGGAATTGA AAGTGACACCG TTTCCTCCAG AAATTGATTT GGGAAATAT 12540
AAACTCTCC CAGAATAACCC AGGCCTCCTC TCTGAGGTCC AGGAGGAAA AGGCATCAAG 12600
46/75

FIG. 8Q

TATAAGTTG AAGTCTACGA GAAGAAAGAC TAACAGGAAG ATGCTTTCAA GTTCTCTGCT 12660
CCCTCCTAA AGCTATGCAT TTTATAAGA CCATGGACT TTTGCTGGCT TTAGATCAGC 12720
CTCGACTGTG CCTTCCTAGTT GCCAGCCATC TGTGTTGC CCCTCCCCG TGCCTTCCTT 12780
GACCCTGGAA GGTGCCACTC CCACTGTCCCT TTCCTAATAA AATGAGGAAA TTGCATCGCA 12840
TTGTCTGAGT AGGTGTCATT CTATTCTGGG GGGTGGGGTG GGGCAGGACA GCAAGGGGA 12900
GGATTGGAA GACAATAGCA GGCATGCTGG GGATGCGGTG GGCTCTATGG AACCAGCTGG 12960
GGCTCGAAGC GGCGCCCAT TTGCTGGTG GTCAGATGCG GGATGGCGTG GGACGGGGCG 13020
GGGAGGGTCA CACTGAGGTT TTCCGCCAGA CGCCCACTGCT GCCAGGGCCT GATGTGCCCG 13080
GCTTCTGACC ATGCGGTGCC GTTCGGTTGC ACTACGGCTA CTGTGAGCCA GAGTTGCCCG 13140
GCGCTCTGG GCTGGGTAG TTCAATCAACT GTTACCTTG TGACCGACA 13200
TCCAGAGGCA CTTCACCGCT TGCCAGGGC TTACCATCCA GCGCCACCAT CCAGTGCAGG 13260
AGCTCGTTAT CGCTATGACG GAACAGGTAT TCGCTGGTCA CTTCGATGGT TTGCCGGAT 13320
AACGGAACT GGAAAAACTG CTGCTGGTGT TTTGCTTCGG TCAGGGCTGG ATGGGGGTG 13380
CGGTGGCAA AGACCAAGACG GTTCATACAG AACTGGCGAT CGTTCGGGGT ATCGCCAAA 13440

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FIG. 8R

TCACCGCCGT AAGCCGACCA CGGGTTGCCG TTTCATCAT ATTAATCAG CGACTGATCC 13500
 ACCCAGTCCC AGACGAAGCC GCCCTGTAAA CGGGATACT GACGAAACGC CTGCCAGTAT 13560
 TTAGCAGAAC CGCCAAGACT GTTACCCATC GCTGGGGCGT ATTGCAAAG GATCAGCGGG 13620
 CCGTCTCTC CGGGTAGCGA AAGCCATT TTGATGGACC ATTCGGGAC AGCCGGGAAG 13680
 GGCTGGTCTT CATCCACGCC CGCGTACATC GGGCAAATAA TATCGGTGGC CGTGGTGTG 13740
 GCTCCGCCG CTTCATACTG CACCGGGCGG GAAGGATCGA CAGATTGAT CCAGCGATAC 13800
 AGGGGTCTG GATTAGGCC GTGGCCTGAT TCATTCCCCA GCGACCAAGAT GATCACACTC 13860
 GGGTGATTAC GATCGCGCTG CACCATTGCG GTTACGGCGTT CGCTCATCGC CGGTAGGCCAG 13920
 CGCGGATCAT CGGTAGACG ATTCATGGC ACCATGCCGT GGGTTCAAT ATTGGCTTCA 13980
 TCCACCACAT ACAGGGCGTA GCGGTCCAC AGCGTGTACC ACAGGGGATG GTTGGGATAA 14040
 TGCCAACAGC GCACGGCGTT AAAGTTGTTC TGCTTCATCA GCAGGATATC CTGCACCATC 14100
 GTCTGCTCAT CCATGACCTG ACCATGCAGA GGATGATGCT CGTGACGGTT AACGCCCTCGA 14160
 ATCAGCAACG GCTTGGCGTT CAGCAGCAGC AGACCAATT CAATCCGCAC CTCGGGAAA 14220
 CCGACATCGC AGGCTTCTGC TTCAAATCAGC GTGCCGGCGG CGGTGTGCAG TTCAACACC 14280

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FIG. 8S

GCACGATAGA GATTGGGGAT TTGGGGCTC CACAGTTTCG GGTTTTCGAC GTTCAGACGC
AGTGTGACGC GATCGGCATA ACCACCACGC TCATCGATAA TTTCACCGCC GAAAGGGCGCG 14340
GTGCCGCTGG CGACCTGCGT TTCACCCCTGC CATAAAGAAA CTGTTACCCG TAGGTAGTCA 14400
CGCAACTCGC CGCACCATCTG AACTTCAGGCC TCCAGTACAG CGCGGCTGAA ATCATCATT 14460
AAGCGAGTGG CAACATGGAA ATCGCTGATT TGTGTAGTCG GTTATGCAG CAACGAGACG 14520
TCACGGAAA TGCCGCTCAT CGGCCACATA TCCTGATCTT CCAGATAACT GCCGTCACTC 14640
CAACGCAGCA CCATCACCGC GAGGGGGTTT TCTCCGGGC GTAAAAATGC GCTCAGGTCA 14700
AATTCAAGACG GCAAACGACT GTCCTGGCCG TAACCGACCC ACGCCCCGTT GCACCCACAGA 14760
TGAAACGCC AGTTAACGCC ATCAAATA ATTTCGGTCT GGCCCTTCCTG TAGCCAGCTT 14820
TCATCAACAT TAAATGTGAG CGAGTAACAA CCCGTGGAT TCTCCGTGGG AACAAACGGC 14880
GGATTGACCG TAATGGATA GGTTACGTTG GTGTAGATGG GCGCATCGTA ACCGTGCATC 14940
TGCCAGTTG AGGGGACGAC GACAGTATCG GCCTCAGGAA GATCGCACTC CAGCCAGCTT 15000
TCCGGCACCC CTTCTGGTGC CGGAAACCAAG GCAAAGGGCC ATTCCGCATT CAGGCTGGCC 15060
AACTGTTGGG AAGGGCGATC GGTGGGGGCC TCTTCCGCTAT TACGCCAGCT GGCGAAAGGG 15120

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FIG. 8T

GGATGTGCTG CAAGGCCATT AAGTTGGTA ACGCCAGGGT TTTCCCAGTC ACGACGTTGT 15180
AAACGACTT ATCCGTCGA GGGGCTGCCT CGAAGCAGAC GACCTTCCGT TGTGCAGCCA 15240
GCGCGCTG CGCCGGTGCCT CACAATCGTG CGCGAACAAA CTAACCAGA ACAATTATA 15300
CCGGCGCAC CGCCGCCACC ACCCTCTCCC GTGCCCTAACCA TTCCAGCGCC TCCACCCACCA 15360
CCACCCAT CGATGTCTGA ATTGCCGGCC GCTCCACCAA TGCCGACGGA ACCTCAACCC 15420
GCTGCACCT TAGACGACAG ACAACAATTG TTGGAAGCTA TTAGAAACGA AAAAAATCGC 15480
ACTCGTCTCA GACCGGTCAA ACCAAAAACG GGGCCGGAAA CCAGTACAAT AGTTGAGGGTG 15540
CCGACTGTGT TGCCCTAAAGA GACATTTGAG CCTAAACCGC CGTCTGCATC ACCGCCACCA 15600
CCTCCGCTC CGCCTCCGCC GCCAGCCCCG CCTGCCCTC CACCGATGGT AGATTATCA 15660
TCAGCTCCAC CACCCGCC ATTAGTAGAT TTGCCGTCTG AAATGTTACC ACCGCCCTGCA 15720
CCATCGCTT CTAACCGTGT GTCTGAATT AAATCGGGCA CAGTTAGATT GAAACCCGCC 15780
CAAAACGCC CGCAATCAGA AATAATTCAA AAAAGCTCAA CTACAAATTG ATCGGGGAC 15840
GTGTTAGCCG ACACAAATTAA TAGGGCTCGT GTGGCTATGG CAAAATCGTC TTGGAAAGCA 15900
ACTTCTAACG ACGAGGGTTG GGACGACGAC GATAATCGGC CTAATAAAGC TAACACGCC 15960

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FIG. 8U

GATGTTAAAT ATGTCCAAGC TACTAGTGGT ACCGGCTTGGC AGAACATATC CATCGCGTCC 16020
 GCCATCTCCA GCAGCCGCAC CGGGCGCATC TCGGGCAGCG TTGGGTCCCTG GCCACGGGTG 16080
 CGCATGATCG TGCTCCTGTC GTTGAGGACC CGGCTAGGCT GGGGGGTTG CCTTACTGGT 16140
 TAGCAGAATG AATCACCGAT ACGCGAGCGA ACGTGAAGGCC ACTGCTGCTG CAAAACGTCT 16200
 GCGACCTGAG CAACAACATG AATGGTCTTC GGTTCCCGTG TTTCGTAAG TCTGGAAACG 16260
 CGGAAGTCAG CGCCCTGCAC CATTATGTTTC CGGATCTGCA TCGCAGGATG CTGCTGGCTA 16320
 CCCTGTGGAA CACCTACATC TGTATTAACG AAGCGCTGGC ATTGACCCCTG AGTGATTTT 16380
 CTCTGGTCCC GCCGCATCCA TACCGCCAGT TGTTTACCCCT CACAAACGTTTC CAGTAACCGG 16440
 GCATGTTCAT CATCAGTAAC CCGTATCGTG AGCATCCTCT CTCGTTTCAT CGGTATCATT 16500
 ACCCCCATGA ACAGAAATCC CCCTTACACCG GAGGCATCAG TGACCAAACA GGAAAAAAC 16560
 GCCCTTAACA TGGCCCGCTT TATCAGAAGC CAGACATTAA CGCTTCTGGA GAAACTCAAC 16620
 GAGCTGGACG CGGATGAACA GGCAGACATC TGTGAATCGC TTCACGACCA CGCTGATGAG 16680
 CTTACCGCA GCTGCCCTCGC GCGTTCCGGT GATGACGGGT AAAACCTCTG ACACATGGCAG 16740
 CTCCGGAGA CGGTACACAGC TTGTCGTAA GCGGATGCCG GGAGCAGACA AGCCCGTCAG 16800

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FIG. 8V

GGCGCGTCAG CGGGTGTGG CGGGTGTGG CGGGCAGCCA TGACCCAGTC ACGTAGCGAT 16860
AGCGGAGTGT ATACTGGCTT AACTATGCCG CATCAGAGCA GATTGTAATG AGAGTGCACC 16920
ATATGCGGTG TGAAATAACCG CACAGATGCG TAAGGAGAAA ATACCGCATC AGGGCCTTT 16980
CGGCTTCCTC GCTCACTGAC TCGGTGCGCT CGGTCTTCC GCTGGGGCGA GCGGTATCAG 17040
CTCACTCAA GGCGGTAATA CGGTTATCCA CAGAACATCAGG GGATAACGCA GGAAAGAACAA 17100
TGTGAGCAA AGGCCAGCAA AAGGCCAGGA ACCGTAaaaa GGCCGGCGTTG CTGGCGTTT 17160
TCCATAGGCT CCGCCCCCT GACGAGGCATC ACAAAATCG ACGCTCAAGT CAGAGGTGGC 17220
GAAACCCGAC AGGACTATAA AGATACCAGG CGTTCCCCC TGGAAAGCTCC CTCGTGCGCT 17280
CTCCTGTTCC GACCCCTGCCG CTTACCGGAT ACCTGTCCGC CTTTCTCCCT TCAGGGAAAGCG 17340
TGGCGCTTTC TCATAGCTCA CGCTGTAGGT ATCTCAGTTTC GGTGTAGGT CTTGGCTCCA 17400
AGCTGGGCTG TGTGCACGAA CCCCGGTTT AGCCCCGACCC CTGCGCCCTTA TCCGGTAACT 17460
ATCGTCTTGA GTCCAACCCG GTAAGACACG ACTTATCGCC ACTGGCAGCA GCCCACTGGTA 17520
ACAGGATTAG CAGAGGGAGG TATGTAGGCG GTGCTACAGA GTTCTTGAAAG TGGTGGCCTA 17580
ACTACGGCTA CACTAGAAGG ACAGTATTG GTATCTGCGC TCTGCTGAAG CCAGTTACCT 17640

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FIG. 8W

TCGGAAAAAG AGTTGGTAGGC TCTTGATCCG GCAAACAAAC CACCGCTGGT AGCGGGTGGTT 17700
TTTTGTTTG CAAGCAGCAG ATTACGCGCA GAAAAAAAGG ATCTCAAGAA GATCCTTTGA 17760
TCTTTCTAC GGGGTCTGAC GCTCAGTGGA ACGAAAACCT ACGTTAAGGG ATTTTGGTCA 17820
TGAGATTATC AAAAGGATC TTACACCTAGA TCCTTTAAA TTAAAATGA AGTTTTAAAT 17880
CAATCTAAAG TATATATGAG TAAACTTGGT CTGACAGTTA CCAATGCTTA ATCAGTGAGG 17940
CACCTATCTC AGCGATCTGT CTATTTCGTT CATCCATAGT TGCCCTGACTC CCCGTCGTGT 18000
AGATAACTAC GATAACGGGAG GGCTTACCAT CTGGCCCCAG TGCTGCAATG ATACCGGAG 18060
ACCCACGCTC ACCGGCTCCA GATTATCAG CAATAAACCA GCCAGCCGGA AGGGCCGAGC 18120
GCAGAAGTGG TCCTGCAACT TTATCCGCCT CCATCCAGTC TATTAATTGT TGCCGGGAAG 18180
CTAGACTAAG TAGTTGCCA GTTAATAGTT TGGCAACGTT TGTTGCCATT GCTGGCAGGCA 18240
TCGTGGTGTAC ACGCTCGTCA TTTGGTATGG CTTCATTCAAG CTCCGGTTCC CAACGATCAA 18300
GGCGAGTTAC ATGATCCCCC ATGTTGTGCA AAAAGGGT TAGCTCCTTC GGCTCCTCGA 18360
TCGTGGTGTAC AAGTAAGTTG GCCGGCAGTGT TATCACTCAT GGTTATGGCA GCACTCGCATA 18420
ATTCTCTTAC TGTCAATGCCA TCCGTAAGAT GCTTTTCTGT GACTGGTGAG TACTCAACCA 18480

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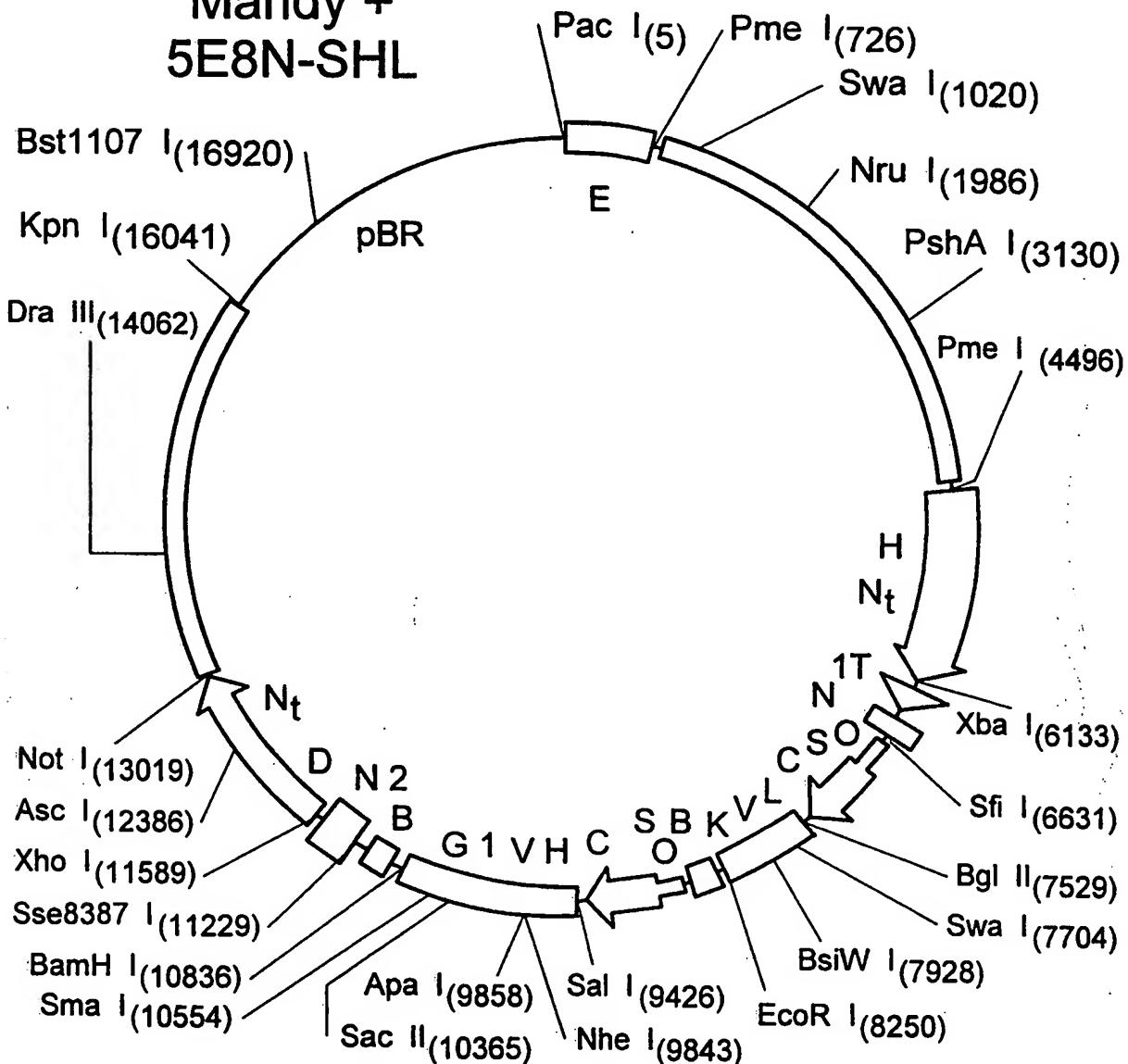
FIG. 8X

AGTCATTCTG AGAATAGTGT ATGCCGGAC CGAGTTGCTC TTGCCCGGCG TCAACACGGG 18540
ATAATCCGC GCCCACATAGC AGAACTTAA AAGTGCTCAT CATTGGAAA CGTTCTCGG 18600
GGCGAAACT CTCAAGGATC TTACCGCTGT TGAGATCCAG TTGGATGTA CCCACTCGTG 18660
CACCCAACTG ATCTTCAGCA TCTTTACCT TCACCGCGT TTCTGGGTGA GCAAAACAG 18720
GAAGGCAAAA TGCCGCAAA AAGGGAAATAA GGGCGACACG GAAATGTTGA ATACTCATAC 18780
TCTCCTTT TCAATATTAT TGAAGCATT ATCAGGGTTA TTGTCTCATG AGCGGATACA 18840
TATTGAAATG TATTTAGAAA AATAAACAAA TAGGGGTTCC GCGCACATT CCCGAAAAG 18900
TGCCACCTGA CGTCTAAGAA ACCATTATTA TCATGACATT AACCTATAA AATAGGCGTA 18960
TCACGAGGCC CTTTCGTCTT CAAGAA 18986

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FIG. 9

Mandy +
5E8N-SHL



Nt D = Inactive Dihydrofolate reductase

E = CMV and SV40 enhancers

Nt H = Inactive *Salmonella* Histidinol Dehydrogenase

T = Herpes Simplex thymidine kinase promoter and polyoma enhancer

C = Cytomegalovirus promoter/enhancer

N1 = Neomycin phosphotransferase exon 1

$K \equiv$ Human kappa constant

VL = Variable light chain anti-CD23 primate 5E8 and leader

VH = Variable heavy chain anti-CD-23 primate 5E8- and leader

B = Bovine growth hormone polyadenylation

M2 = Neomycin phosphotransferase exon 2

G1 = Human Gamma 1 constant

GT - Human Gamma 1 constant
Mandy cut Yba1 Yba1 and ligated to Yba1 Yba1 fragment

Mandy cut Xbal Xho I and lig from YKC1+CD23 E53N R111

from XKGT1+CD23 SE8N-SHL
Map by Mitchell Poff. Generated by Karen McEachan. 06/22/27. 18,225 L.

Map by Mitchell Reff Constructed by Karen McLachlan 06/26/9
Nanulators = Afill, Avill, Hindfill, Prol, Seal, Pmll, Parll, Scll, Sfl

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10	TTAATTAAAGG	20	GGCGGAGAAAT	30	GGGGGGAACT	40	AGGGGGAGTT	50	TGGGGGGGA	60	TAGGGGGGG
80	ACTATGGTTG	90	CTGACTAATT	100	GAGATGCATG	110	CTTTCGCATAC	120	TGGGGAGCCT	130	GGGGGACTTTTC
150	CACACCTGGT	160	TGCTGACTAA	170	TTGAGATGCA	180	TGCTTGCAT	190	GCTTCTGCCT	200	CTGGGGAGC
220	TCCACACCT	230	AACTGACACA	240	CATTCCACAG	250	AATTAATTCC	260	AATAGTTATT	270	AATTACGGGG
290	TCATTAGTTC	300	ATAGCCCATA	310	TATGGAGTTC	320	CGCGTTACAT	330	AACTTACGGT	340	CCTGGCTGAC
360	CGCCCAACGAA	370	CCCCGGCCA	380	TTGACGTCAA	390	TAATGACGTA	400	AAATGGCCCG	410	TAGGGACTTT
430	CCATTGACGT	440	CAATGGGTGG	450	AGTATTACG	460	GTAAACTGCC	470	TACATCAAGT	480	GTATCATATG
500	CCAAGTACGC	510	CCCTTATTGA	520	CGTCAATGAC	530	GGTAAATGGC	540	TTATGCCAG	550	GTACATGACCT
570	TATGGACTT	580	TCCTACTGG	590	CAGTACATCT	600	ACGTATTAGT	610	ACCATGGTGA	620	TGCGGGTTTG
640	GCAGTACATC	650	AATGGCGGTG	660	TGACTCAGG	670	CATGCTATT	680	TTAACGTGT	690	CATTGACGTC
710	AATGGGAGTT	720	TGTTTGAAG	730	GATAGGGTT	740	GGATTTCCAA	750	GTCTCCACCC	760	TTACGTGAG
780	TCAATTGTAC	790	ACTAACGACA	800	CTGTTAAC	810	GCCAGCTTA	820	TTTTGAACGA	830	CGTCGAACCT
850	TTATTACAAA	860	ACAAAACACA	870	GTGATGAAAG	880	AAATACAAAA	890	900	910	GCAAGTTTG
920	TGGCGTTGAG	930	CGAAAATCCA	940	AACGAATATC	950	GACAAAGCTA	960	ACAAGATTG	970	980
					TTAGATAAGTC		CAGCCATCGG	TTGGGAAAAA	CAACCCTTGT		TTGAAACTAA

FIG. 10A

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990	TCGAAACCTA	1000	TTTACAAAT	1010	CTATTGAGGA	1020	TTAACATATT	1030	AAAATTCAGAT	1040	TGAAAATCAT	1050
1060	TTGATTTCG	1070	CTCTAACATA	1080	CCACCCCTAAA	1090	GATTATAAAT	1100	TTAACAAATAC	1110	ATCAGCAACT	1120
1130	ATATATTGAT	1140	AGACATTTCC	1150	AGTTTGTGAT	1160	ATTAGTTTGT	1170	GGTCTCATT	1180	TTATTTTAA	1190
1200	CAACAAACAA	1210	CTGCTCGCAG	1220	ACAATAGTAT	1230	AGAAAAGGGA	1240	GGTGAACTGT	1250	CGGTTCGTAC	1260
1270	AACATTTGG	1280	AAAGTTATGT	1290	TAATCCGGTG	1300	CTGCTAAAAA	1310	TTTGTGTTAA	1320	GAAGCTGCCG	1330
1340	ACTATGCCGG	1350	CAACATATTG	1360	TACAAAACCG	1370	ACGATCCAA	1380	TGAACTAGAA	1390	TAATAATTAA	1400
1410	AGCAACACAC	1420	TCCGAAGAAC	1430	TACCAGAAAA	1440	TAGCACTGTT	1450	GAAAAACTAT	1460	GCGCAGCGGT	1470
1480	ACTATACACC	1490	CCATTAAAAA	1500	AGACATATAT	1510	ATTATGACA	1520	TACTCTATAC	1530	GATAGATAACA	1540
1550	TATATGGATA	1560	CGATAAAC	1570	TATGTTAATT	1580	TTTATGAGGA	1590	AAAGAGAAAGG	1600	AATACGAAGA	1610
1620	AGAAGACGAC	1630	AAGGCCTCA	1640	GTTTATGTGA	1650	AAATAAAATT	1660	AAATTAACGT	1670	TGAATCATTT	1680
1690	GAAAATGATT	1700	TTAAATATTA	1710	CCTCAGCGAT	1720	TATAACTACG	1730	TATAGATAAT	1740	ACTACAAATG	1750
1760	TTCTTGTGC	1770	GTTGGTTTG	1780	TATCGTTAAT	1790	AAAAAACAAA	1800	TTTGACATT	1810	TATTATTCAA	1820
1830	TAATTACAAA	1840	ACCGCTTGAG	1850	TTGCCAGCAA	1860	ACGGACAGAG	1870	TTTGTGTTG	1880	AGAGTTGTTG	1890
1900	ATTCAATTGTT		TAGGATTGAG	1910	1920	TCACCGAAGT	1930	1940	TCATGCCAGT	1950	CCAGCGTTT	1960
			TGCCTCCCTG		CTGGGGTTT						TGCAGCAGAA	

FIG. 10B

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1970	AAGCCGCCGA	CCTCGGTTG	1980	1990	2000	2010	2020	2030
2040	TTGGAGGTC	GCAAATCGG	2050	2060	2070	2080	2090	2100
2110	CGGTGATACA	TATCCAGCCA	2120	2130	2140	2150	2160	2170
2180	CGGCTAACGT	ATCCACGCCG	2190	2200	2210	2220	2230	2240
2250	TTCTTTTCC	AGTACCTCT	2260	2270	2280	2290	2300	2310
2320	AGGCACAGCA	CATCAAAGAG	2330	2340	2350	2360	2370	2380
2390	TGATCGGACG	CGTCGGGTCG	2400	2410	2420	2430	2440	2450
2460	CGGACGGTA	TCCGGTTCGT	2470	2480	2490	2500	2510	2520
2530	AGCTCTTAA	TCGGCTGTAA	2540	2550	2560	2570	2580	2590
2600	TGGCTTGT	GCCCGTTCG	2610	2620	2630	2640	2650	2660
2670	CACCAAGATG	CCATGTTCAT	2680	2690	2700	2710	2720	2730
2740	GAGTTGGCCC	CAATCCAGTC	2750	2760	2770	2780	2790	2800
2810	GCAAGTCCGC	ATCTTCATGA	2820	2830	2840	2850	2860	2870
2880	GCCCTTCACT	GCCACTGACC	2890	2900	2910	2920	2930	2940

FIG. 10C

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2950	GTGACGCACA	2960	GTTCATAGAG	2970	ATAACCTTCA	2980	CCCGGTTGCC	2990	AGAGGGTGGG	3000	TGCAAAGTCC
3020	CGCTAGTGCC	3030	GCAACCACCT	3040	GTTGATCCGG	3050	ATCACGGCAGT	3060	TCAACGGCTGA	3070	CATCACCATT
3090	GGCCACCACC	3100	TGCCAGTCAA	3110	CAGACCGGTG	3120	GTTACAGTCT	3130	TGGCGACAT	3140	GGTGATATCG
3160	TCCACCCAGG	3170	TGTTGGCGT	3180	GGTAGAGGC	3190	ATTACGCTGC	3200	GATGGATTCC	3210	AAGAAATCAT
3230	GGAAAGTAAGA	3240	CTGCTTTTC	3250	TTGCCGTTTT	3260	CGTGGTAAT	3270	GGCATAGTTA	3280	TCTGCCAGTT
3300	CAGTTCGTTG	3310	TTCACACAAA	3320	CGGTGATACC	3330	CCTCGACGGA	3340	GGCGGGATAG	3350	ACTATGAAGA
3370	AGTGTTCGTC	3380	TTCGTCCCAG	3390	TAAGCTATGT	3400	CTCCAGAAATG	3410	TCAAGGGCTT	3420	3430
3440	GGTCGCTTCC	3450	GGATTGTTA	3460	CATAACCGGA	3470	TAGCCATCCA	3480	TCTTGTCAA	3490	TCTCTCTGA
3510	TTAACGCCCA	3520	GGGTTTCCC	3530	GGTATCCAGA	3540	CATAATCATA	3550	GGCTCTCTGA	3560	3570
3580	CCGGCCCCGG	3590	TTTATCATCC	3600	CCCTCGGGTG	3610	TAATCAGAAT	3620	AAATGAAACA	3630	ACTTTACCGA
3650	TTGTCGTATC	3660	CCTGGAAGAT	3670	GGAAAGCGTT	3680	TCCCAACCCT	3690	GTCTCAGTGA	3700	3710
3720	CCAGAAGCAA	3730	TTTCGTGTA	3740	ATTAGATAAA	3750	TGTTTGTGTT	3760	CTTTGGCG	3770	AGGTGGCCCC
3790	ATAGGGTTGG	3800	TACTAGCAAC	3810	GCACTTTGAA	3820	CAATCAGAGT	3830	AAGAATGAAA	3780	3790
3860	AAATCTATAC	3870	ATTAAGACGA	3880	CTCGAAATCC	3890	CTGAAGGGAT	3900	GCTCTTCTTC	3910	3920

FIG. 10D

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3930	TGATGGAATG	3940	GAACAAACACT	3950	TAAAATCGCA	3960	GTATCCGGAA	3970	TGATTGATT	3980	GCCAAAAATA	3990	GGATCTCTGG
4000	CATGCGAGAA	4010	4020	GCAGTTCTAT	4030	GGGGAAAGGGC	4040	CACACCCTTA	4050	GGTAACCCAG	4060	TAGATCCAGA	
4070	GGATTGTTT	4080	4090	AAAGGACTCT	4100	GGTACAAAT	4110	CGTATTCACT	4120	AAAACGGGA	4130	GGTAGATGAG	
4140	ATGTGACGAA	4150	4160	GAUTGAAATC	4170	CCTGTAATC	4180	CGTTTAGAA	4190	TCCATGATAA	4200	TAATTCTG	
4210	GATTGGT	4220	4230	GCACGTTCAA	4240	AATTTCGC	4250	AACCCCTTT	4260	GGAAACAAA	4270	CACTACGGTA	
4280	GGCTGCGAAA	4290	4300	GTTGAGCAAT	4310	TCACGTTCAT	4320	TATAATGTC	4330	GCAAACGTGCAA	4340		
4350	CTCCGATAAA	4360	4370	AACACGGCC	4380	TAAAGATTG	4390	GGTCGGGGC	4400	CGACGATTCT	4410		
4420	GTGATTGTA	4430	4440	ATCGTTTCA	4450	AAGAGAGTTT	4460	TCACTGCATA	4470	CGACGATTCT	4480		
4490	TACAGCCGG	4500	4510	GGCGGGCTT	4520	AGCTTCTGCC	4530	ACATTTCGAA	4540	GTATTCCGGC	4550		
4560	CAGCGTGC	4570	4580	TCCGGCGATT	4590	CAATACCCCTG	4600	ACAGCTGTAG	4610	CCCTGAACAG	4620		
4630	TGGATAATGT	4640	4650	GGTGACGATG	4660	TCCGCCTCTG	4670	CCGGACGGTC	4680	AGCGATATTTC	4690		
4700	GACAGCGCTA	4710	4720	CTGAAGAGAT	4730	CCCGCCGCC	4740	AAATTGATA	4750	AAACAGAAAGT	4760		
4770	GGGATGACCG	4780	4790	AAATATTGAA	4800	GGCGCGGTGA	4810	4750	4820	ATTAAAACAG	4830		
4840	AAACCCAGCC	4850	4860	AGGCAGGCGT	4870	ACGTTCCATT	4880	TGAGCGACGA	4890	GTAGATGTGG	4900		

FIG. 10E

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4910	CGGCTGGCT	4920	CCGCCTTCT	4930	CAACGGTGT	4940	GATGGCTGGCT	4950	ACGCCGGCG	4960	GCATTGGGG	4970
4980	GTGGTTCTGT	4990	GCTCGCCGCC	5000	GCCCATCGCT	5010	GATGAAATCC	5020	TCTATGCGGC	5030	GGCGTGCAGG	5040
5050	AAATCTTTAA	5060	CGTCGGGGC	5070	GCGCAGGGGA	5080	TTTGCCTGCT	5090	GGCCTTCGGC	5100	TACCGAAAGT	5110
5120	GGATAAAATT	5130	TGGCCCCG	5140	TGTAAACCGCTT	5150	TGTAAACCGAA	5160	AGCGAGTC	5170	GCGTCTCGAC	5180
5190	GGCGCGGCTA	5200	TCGATATGCC	5210	GGCGGGCGG	5220	TCTGAAGTAC	5230	AGGTCAAGCCA	5240	GCAACACCGG	5250
5260	ATTTCGTCGC	5270	TTCTGACCTG	5280	CTCTTCCCAGG	5290	TGGTGATCGC	5300	AGACAGGGGC	5310	TGCTGACGCC	5320
5330	TGATGCTGAC	5340	ATTGCCCGCA	5350	AGGTGGCGGA	5360	CTGAGCACGG	5370	CAGGTGATCC	5380	GCGGCCATCT	5390
5400	ACCGCCGGC	5410	AGGCCCTGAG	5420	CGCCAGTCGT	5430	CTGATTGTGA	5440	CGTAACCTGG	5450	GCGCAGTGC	5460
5470	CTAACATCGTA	5480	TGGGGGGAA	5490	CACTTAATCA	5500	TCCAGACGGG	5510	GATTTGGTGG	5520	ATGCGATTAC	5530
5540	CAGCGCAGGC	5550	TCGGTATTTC	5560	TCGGCGACTG	5570	GTCGCCGGAA	5580	TCCGCCGGTG	5590	CGGAACCAAC	5600
5610	CATGTTTAC	5620	CGACCTATGG	5630	CTATACTGCT	5640	ACCTGTTCCA	5650	AGGGATTTC	5660	CAGAAACGGA	5670
5680	TGACCGTTCA	5690	GGAACTGTCTG	5700	AAAGCGGGCT	5710	TTTCCGGCTCT	5720	GGCATCAACC	5730	TGGGGCGGGC	5740
5750	AGAACGGTCTG	5760	ACCGCCATA	5770	AAAATGCCGT	5780	GACCCCTGCC	5790	ATTGAAACAT	5800	AGCATGAGCA	5810
5820	CTGAAAACAC	5830	TCTCAGCGTC	5840	GCTGACTTAG	5850	CCCGTGAAAA	5860	TCAAGGCC	5870	AGACATGGAT	5880

FIG. 10F

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5890	5900	5910	5920	5930	5940	5950
AAGATACATT	GATGAGTTG	GACAAACCAC	AACTAGAAC	CAGTGAAAAA	AATGCTTAT	TTGTGAAATT
5960	5970	5980	5990	6000	6010	6020
TGTGATGCTA	TTGCCTTATT	TGTAACCATT	ATAAGCTGCA	ATAAAACAAGT	TAACAAACAC	AATTGCATTC
6030	6040	6050	6060	6070	6080	6090
ATTTTATGTT	TCAGGGTCAG	GGGGAGGTGT	GGGAGGTTT	TTAAAGCAAG	TAACACCTCT	ACAAATGTGG
6100	6110	6120	6130	6140	6150	6160
TATGGCTGAT	TATGATCTCT	AGGGCCGCC	CTCGACGGCG	CGTCTAGAGC	AGTGTGGTTT	TCAAGAGGAA
6170	6180	6190	6200	6210	6220	6230
GCAAAAAGCC	TCTCCACCC	GGCCTGGAAT	GTTTCCACCC	AATGTCGAGC	AGTGTGGTTT	TGCAAGAGGA
6240	6250	6260	6270	6280	6290	6300
AGCAAAAAGC	CTCTCCACCC	AGGCCTGGAA	TGTTTCCACC	CAATGTCGAG	CAAACCCCGC	CCAGCGTCTT
6310	6320	6330	6340	6350	6360	6370
GTCATTGGCG	AATTGGAACCA	CCCATATGCA	GTCGGGGCGG	CGCGGGCCCA	GGTCCACTTC	GCATATTAAG
6380	6390	6400	6410	6420	6430	6440
GTGGCCGCTG	TGGCCTCGAA	CACCGAGCGA	CCCTGCAGCC	AATATGGGAT	CGGCCATTGA	ACAAGATGGA
6450	6460	6470	6480	6490	6500	6510
TTGCACGCAG	GTTCTCCGGC	CGCTTGGGTG	GAGAGGCTAT	TCGGCTATGA	CTGGCACCAA	CAGACAATCG
6520	6530	6540	6550	6560	6570	6580
GCTGCTCTGA	TGCCGCCGTG	TTCCGGCTGT	TTCCGGGG	GCGCCCCGGTT	CTTTTTGTCA	AGACCGACCT
6590	6600	6610	6620	6630	6640	6650
GTCCGGTGCC	CTGAATGAAC	TGCAAGTAAG	TGCGGCCGTC	GATGGCCGAG	GCGGCCCTCGG	CCTCTGCATA
6660	6670	6680	6690	6700	6710	6720
AATAAAAAAA	ATTAGTCAGC	CATGCATGGG	GCGGAGAAATG	GGCGGAACGTG	GGGGAGTTA	GGGGGGGGAT
6730	6740	6750	6760	6770	6780	6790
GGCGGGAGTT	AGGGCGGGGA	CTATGGTTGC	TGACTAATTG	AGATGCATGC	TTTGCTACT	TCTGCTGCT
6800	6810	6820	6830	6840	6850	6860
GGGGAGGCC	GGGACTTTCC	ACACCTGGTT	GCTGACTAAT	TGAGATGCAT	GCTTGGCTA	CTTCTGGCTG

FIG. 10G

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6870	CTGGGAGCC	TGGGACTT	6890	CCACACCCTA	ACTGACACAC	6900	ATTCCACAGA	6910	ATTAATTCCC	6920	CTAGTTATTA
6940	ATAGTAATCA	ATTACGGGGT	6950	CATTAGTTCA	TAGCCCATAT	6970	ATGGAGTTCC	6980	GCGTTACATA	6990	ACTTACGGTA
7010	AATGGCCCGC	CTGGCTGACC	7020	GCCCAACGAC	CCCCGCCCAT	7040	TGACGTCAAT	7050	AATGACGTAT	7060	GTTCCCATAG
7080	TAACGCCAAT	AGGGACTTTC	7090	CATTGACGTC	AATGGGTGGA	7110	GTATTTCAGG	7120	TAAACTGCC	7130	ACTTGGCAGT
7150	ACATCAAAGTG	TATCATATGC	7160	CAAGTACGCC	CCCTATTGAC	7180	GTCAATGACG	7190	GTAATGGCC	7200	CGCCTGGCAT
7220	TATGCCCAGT	ACATGACCTT	7230	ATGGGACTTT	CCTACCTGCC	7240	AGTACATCTA	7250	CGTATTAGTC	7270	ATCGCTATTA
7290	CCATGGTGAT	GCGGTTTGG	7300	CAGTACATCA	ATGGCGTGG	7310	ATAGGGTTT	7330	GACTCACGGG	7340	GATTTCCAAG
7360	TCTCCACCCC	ATTGACGTCA	7370	ATGGGAGTTT	GTGTTGGCAC	7380	CAAATCAAC	7390	GGGACTTTCC	7400	AAAATGTCGT
7430	AACAACCTCCG	CCCCATTGAC	7440	GCAAATGGGC	GGTAGGGCGTG	7450	TACGGTGGGA	7470	GGTCTATATA	7480	AGCAGAGGCTG
7500	GGTACCGTGA	CCGTCAGATC	7510	GCCTGGAGAC	GCCATCACAG	7520	7530	7540	7550	GGGTCCCCGC	7560
7570	TCAGGCTCTG	GGGCTCCTC	7580	TGCTCTGGCT	CCCAGGTGCC	7590	AGATGTGACA	7610	ATGGACATGA	7620	CCAGTCTCCA
7640	TCTTCCCTGT	CTGCATCTGT	7650	AGGGGACAGA	GTCACCATCA	7660	7670	7680	7690	7700	ATTAGTATT
7710	ATTAAATTG	GTATCAGCAG	7720	AAACCAGGAA	AAGCTCCTAA	7730	7740	7750	7760	7770	CCAGTTTGCA
7780	AAGTGGGTC	CCATCAAGGT		TCAGGGCAG	TGGATCTGG	7800	7810	7820	7830	7840	CAGCAGCGT

FIG. 10H

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7850	CAGCCTGAAG	7860	TTTATTGCGAC	7870	CTACAGGTGTT	7880	ATAGTACCCC	7890	TCGGACGTTT	7900	GGCCAAGGGAA
7920	CCAAGGGTGGAA	7930	AATCAAACGT	7940	ACGGGTGGCTG	7950	CACCATCTGT	7960	CCGCCATCTG	7970	ATGAGGCAGTT
7990	GAAATCTGGAA	8000	ACTGCCCTTG	8010	TTGTGTGCCCT	8020	GCTGAATAAC	8030	TTCTATCCCA	8040	GAGAGGCCAA
8060	AAGGTGGATA	8070	ACGCCCTCCA	8080	ATCGGGTAAC	8090	TCCCAGGGAGA	8100	GTGTCACAGA	8110	AGTACAGTGG
8130	CCTACAGGCCT	8140	CAGCAGGCC	8150	CTGACGCTGA	8160	GCAAAGCAGA	8170	GCAGGGACAGC	8180	AAGGACACAGCA
8200	AGTCACCCAT	8210	CAGGGCCTGA	8220	GCTCGCCCGT	8230	CACAAAGAGC	8240	CTACGAGAAA	8250	ACGCCTGCGA
8270	CGTTAACCGGT	8280	TACCAAAC TAC	8290	CTAGACTGGA	8300	TTCGTGACAA	8310	GAGAGTGTGG	8260	AATTCA GATC
8340	CCTCGACTGT	8420	GCCTTCTAGT	8350	TGCCAGCCAT	8360	CTGTTGTTG	8370	TTCAACAGGG	8320	GTATGATCTAC
8410	AGGTGCCACT	8490	CCCAC TGTCC	8430	TTCCCTAATA	8440	CTGTTGTTG	8380	CATGGGGCC	8390	GTGACCTCTTCT
8480	TCTATTCTGG	8560	GGGGTGGGGT	8500	GGGGCAGGAC	8510	AAATGAGGAA	8440	CCCCCTCCCC	8330	GTATGATCTAC
8550	GGGATGCGGT	8620	GGGCTCTATG	8570	GCTTCTGAGG	8580	AGCAAGGGGG	8510	GTGAGTGTGG	8400	GTGACCTCTTCT
8690	TAGGGCGGG	8700	ATGGGGGAG	8630	TTAGGGGGGG	8640	GGACTATGGT	8720	GGGACTAAT	8460	GTGAGTGTGG
8770	CTGGGGAGCC	8770	TGGGGACTTT	8780	CCACACCTGG	8790	TTGCTGACTA	8730	TGAGATGCAT	8450	GTGACCTCTTCT
8760	TACTTCTGCC		CCTGGGGAG		TTCCACACCC		TAACTGACAC		TTGCTGACTA	8740	GTGACCTCTTCT

FIG. 10I

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8830	CCCTAGTTAT	8840	TAATAGTAAT	8850	CAATTACGGG	8860	GTCATTAGTT	8870	CATAGCCCCAT	8880	CCGGGTTACA	8890
8900	TAACCTACGG	8910	AAATGGCCC	8920	GCCTGGCTGA	8930	CGGCCCAACG	8940	ACCCCCGCC	8950	ATAATGACGT	8960
8970	ATGTTCCCAT	8980	AGTAACGCCA	8990	ATAGGGACTT	9000	TCCATTGACG	9010	TCAATGGGTG	9020	GGTAAACTGC	9030
9040	CCACTTGGCA	9050	GTACATCAAG	9060	GCCAAGTACG	9070		9080	GAGTATTAC	9090	CGTAAATGG	9100
9110	CCC GCCCTGGC	9120	ATTATGCCA	9130	TTATGGGACT	9140	CCCCCTATTG	9150	ACGTCAATGA	9160	CGTAAATGG	9170
9180	TCATCGCTGT	9190	TACCATGGT	9200	GGCAGTACAT	9210	TTCCCTACTTG	9220	GCAGTACATC	9230	TACGTATTAG	9240
9250	GGGATTCCA	9260	AGTCTCCACC	9270	ATGGGTTTT	9280	CAATGGGGT	9290	GGATAGCGGT	9300	TTGACTCAGG	9310
9320	CCAAATGTC	9330	GTAACAACTC	9340	CCATTGACGT	9350	CAATGGGAGT	9360	ACCAAATCA	9370	ACGGGACTTT	9380
9390	TAAGCAGAGC	9400	TGGGTACGTG	9410	CGCCCCATTG	9420	ACGCAAATGG	9430	TGTACGGTGG	9440	GAGGTCTATA	9450
9460	TGCTCTTCCT	9470	AACCGTCAGA	9480	TCGCCCTGGAG	9490	ACGCCGTGGA	9500	CATGGGTGG	9510	AGCCTCATCT	9520
9530	GGCAAAGCCT	9540	TGTCGCTGTT	9550	GCTACGGCTG	9560	TCCCTGTCGA	9570	GGGGAGTCTG	9580	GGGGGGGCTT	9590
9600	TACATGGACT	9610	GGGTCCGCCA	9620	CTGAGACTCT	9630	CTGCCAGGC	9640	GGTTCACCTT	9650	CAATAACTAC	9660
9670	ATCCCACATG	9680	GGGGGTCCC	9690	GGCTCCAGGG	9700	TCCGGCTGG	9710	ACGTATTAGT	9720	AGTAGTGGTG	9730
9740	GTTTCTCAA	9750	GTACGCAGAC	9760	TCCGTGAAGG	9770	AGTGGGTCTC	9780	GAGAACGCCA	9790	AGAACACACT	9800
			ATGAACAGGC		TGAGAGCTGA		GGACACGGCT		GTCTTAACT		GAATCAGGG	

FIG. 10J

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9810	TCTGACTCCCT	GGGCCAGGG	9820	9830	ACCGCTGGTC	9840	CAGCTAGCCT	9850	CAAGGGCCA	9860	TCGGTCTTCC
9880	CCCTGGCAC	CTCCCTCCAAG	9890	9900	AGCACCTCTG	9910	GGGCACAGC	9920	GGCCCTGGGC	9930	9940
9950	CCCCGAACCG	GTGACGGTGT	9960	9970	CGTGGAACTC	9980	AGGGCCCTG	9990	TGCACACCTT	10000	AGGACTACTT
10020	CTACAGTCCT	CAGGACTCTA	10030	10040	CTCCCTCAGC	10050	ACCAAGGGCG	10060	CCGGCTGTC	10070	10010
10090	CCTACATCTG	CAACGTGAAT	10100	10110	GCAAGGCCA	10120	GGGGACAAG	10130	CAGCAGCTTG	10140	10080
10160	TGACAAAACT	CACACATGCC	10170	10180	CACCGTGGCC	10190	AGCACCTGAA	10200	GGGGACAG	10210	GGCACCCAGA
10230	CCCCAAAAC	CCAAGGACAC	10240	10250	CCTCATGATC	10260	TCCCGGACCC	10270	CTGAGGTGAC	10280	CCAATCTTG
10300	GCCACGAAGA	CCCTGAGGTC	10310	10320	AAGTTCAACT	10330	GGTAGTGGGA	10340	ATGGAGCTGA	10350	GTGGACGTGA
10370	GCGGGGGAG	GAGCAGTACA	10380	10390	ACAGCACGTA	10400	CCGTGTGGTC	10410	GTGCATAATG	10420	10360
10440	CTGAATGGCA	AGGAGTACAA	10450	10460	GTGCAAGGTC	10470	AGCGTCCTCA	10480	CCGTCTGCA	10490	CCAAGACAAA
10510	CCAAAGCCAA	AGGGCAGCCC	10520	10530	CGAGAACAC	10540	TCCAACAAAG	10550	CCCCATCGAG	10560	10430
10580	GAACCAGGTC	AGCCTGACCT	10590	10600	GGCTGGTCAA	10610	AGGTGTACAC	10620	TCCCAGGATG	10630	CCAGGACTGG
10650	AATGGGCAGC	CGGAGAACAA	10660	10670	CTACAAGACC	10680	AGGCTTCTAT	10690	TCGCCGTGGA	10700	10640
10720	ACAGCAAGCT	CACCGTGGAC	10730	10740	AAGAGCAGGT	10750	ACGCCTCCCC	10760	CGACGGCTCC	10770	10780

FIG. 10K

FIG. 10L

10790	GGCTCTGCAC	AACCACTACA	10800	10810	CGCAGAAAGAG	10820	10830	TCTCCCGGCTG	10840	10850	CGTTAACGGGT
10860	TACCAACTAC	CTAGACTGGA	10870	10880	TTCGTGACCAA	10890	10900	TGATATCTAC	10910	10920	CCTCGACTGT
10930	GCCTTCTAGT	TGCCAGCCAT	10940	10950	CTGTTGTTGC	10960	10970	GTGCCCTCCCT	10980	10990	AGGTGCCACT
11000	CCCACTGTCC	TTTCCTAATA	11010	11020	AAATGAGGAA	11030	11040	ATTGTCTGAG	11050	11060	TCTATTCTGG
11070	GGGGCTGGGT	GGGGCAGGAC	11080	11090	AGCAAGGGGG	11100	11110	AGGCATGCTG	11120	11130	GGGATGCCGT
11140	GGGCTCTATG	GCTTCTGAGG	11150	11160	CGGAAAAGAAC	11170	11180	AGACAATAGC	11190	11200	AGGCCGCTAC
11210	CTCCCTCCT	TTCCCTGCAG	11220	11230	GACGGGGCAG	11240	11250	CGCTAGGTCG	11260	11270	ACGACGGGGC
11280	TAACTCTCTC	AGCTGTGCTC	11290	11300	TCGAGCTTCA	11310	11320	GTGGCTGGCC	11330	11340	GCGAAAGTGCC
11350	TCCTTGGCG	GGGGCAGGAT	11360	11370	CTCACCTTGC	11380	11390	CTGCTATTGG	11400	11410	TGCAATGCCG
11420	CGGCTGCATA	CGCTTGTCC	11430	11440	GGCTACCTGC	11450	11460	TCATGGCTGA	11470	11480	GAGGGAGCAC
11490	GTACTCGGAT	GGAAAGCCGGT	11500	11510	CTTGTGATC	11520	11530	ACATCGCATC	11540	11550	TGGCCAGC
11560	CGAACTGTT	GCCAGGTAAG	11570	11580	TGAGCTCCAA	11590	11600	CATCAGGGGC	11610	11620	GTAGCTTGC
11630	TTCTCAATT	CTTATTTGCA	11640	11650	TTCAAGCTCT	11660	11670	CGGCCAGCTA	11680	11690	GTAGTTGATT
11700	GAGCAAATGC	GTTGCCAAA	11710	11720	TAATGAGAAA	11730	11740	TTAATTAA	11750	11760	CACCAATTCA
					AGGATGCCCTT			AGAGACAGTG			AGATAAGGAC

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11770	CAGAGGGAGT	11780	ACCCAGAGCT	11790	GAGACTCCTA	11800	TGCCAGTGAG	11810	TCCAGGGAGA	11820	AATATGCTTG
11840	TCATCACCGA	11850	AGCCTGATTTC	11860	CGTAGAGCCA	11870	AGGGCCAATC	11880	TGCTCACACA	11890	GGATAGAGAG
11910	GGCAGGAGCC	11920	AGGCAGAGC	11930	ATATAAGGTG	11940	AGTTAGGATC	11950	CACATTGGCT	11960	TCTGACATAG
11980	TTGTGTTGGG	12000	AGCTTGGATA	12000	GCTTGGGGGG	12010	GGGACAGGCTC	12020	AGGGCCTGGGA	12030	AACTTGACGG
12050	CAATCCCTAGC	12060	GTGAAGGGCTG	12070	GTAGGATTTT	12080	ATCCCCGCTG	12090	TCGACCATTG	12100	AACTGCATCG
12120	TCGCCGTGTC	12130	CCAAAATATG	12140	GGGATTGGCA	12150	CCTACCCCTGG	12160	12170	12180	GGAACGAGTT
12190	CAAGTACTTC	12200	CAAAGAATGA	12210	CCACAACCTC	12220	GGTAAACAGA	12230	12240	12250	TATGGTAGG
12260	AAAACCTGGT	12270	TCTCCATTCC	12280	TGAGAAGAAT	12290	CGACCTTTAA	12300	ATCTGGTAGAT	12310	12320
12330	AACTCAAAGA	12340	ACCAACCAGA	12350	GGAGCTCATT	12360	AGGACAGAAAT	12370	TAATATAGTT	12380	CTCAGTAGAG
12400	CCATTAAGAC	12410	TTATTGAACA	12420	ACCGGAATTG	12430	AAGTTTGGAT	12440	GATGCCTTAA	12450	CGTAGGGCGG
12470	CTGTTACCA	12480	GGAAAGCCATG	12490	AATCAACCAG	12500	TAGACATGGT	12510	12520	12460	GGAGGGCAGTT
12540	TGAAAGTGAC	12550	ACGTTTTTC	12560	CAGAAATTGA	12570	ACTCTTGTG	12580	ACAAGGATCA	12590	TGCAGGAATT
12610	CTCTCTGAGG	12620	TCAAGGAGGA	12630	AAAAGGCATC	12640	TATAAACCTTC	12650	12660	12670	CCCAGGGCTC
12680	AAGATGCTTT		CAAGTTCTCT	12700	GCTCCCTCC	12710	TTGAAGTCTA	12720	CGAGAAAGAAA	12730	GACTAACAGG
							TAAAGCTATG		CATTTTTATA		ACTTTTGCTG

FIG. 10M

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12750	GCTTTAGATC	12760	AGCCCTCGACT	12770	GTTGCCCTCTA	12780	ATCTGCCAGCC	12790	TGCCCTTGT	12800	CCGTGCCTTC
12820	CTTGACCCCTG	12830	GAAGGGTCCA	12840	CTCCCCACTGT	12850	CCTTTCCCTAA	12860	AAATTGAGG	12870	GCATTGCTG
12890	AGTAGGTGTC	12900	ATTCTATTCT	12910	GGGGGGTGGG	12920	GTGGGGCAGG	12930	ACAGCAAGGG	12940	GAAGACAAATA
12960	GCAGGCATGC	12970	TGGGATGCC	12980	GTGGGCTCTA	12990	GGGGAAAGA	13000	GGAGGATTGG	13010	GCTCGAAGCG
13030	GCGGCCATT	13040	TCGCTGGTG	13050	TAGATGCCG	13060	ACAGGGCTG	13070	ACCAAGCTGG	13080	13090
13100	TCCGCCAGAC	13110	GCCACTGCTG	13120	CCAGGGCTG	13130	GATGGCGTGG	13140	GAGCGGGCGG	13150	ACTGAGGTTT
13170	CTACCGTAC	13180	TGTGAGCCAG	13190	AGTTGCCGG	13200	CTTCTGACCA	13210	TGCGGTGCG	13220	TTCCGGTTGCA
13240	TTTACCTTGT	13250	GGAGCGACAT	13260	CCAGAGGCAC	13270	CGCTCTCCGG	13280	CAATCAAATG	13290	13300
13310	CAGTGCAGGA	13320	GCTCGTTATC	13330	GCTATGACGG	13340	GGCACCGCCT	13350	TACCATCCAG	13360	CGCCACCATC
13380	AACGGAACTG	13390	GAAAAGACTGC	13400	TTGCTGGTGT	13410	GGCACCCGCT	13420	TTCGATGGTT	13430	13370
13450	GACCAGACCG	13460	TTCATACAGA	13470	ACTGGGGATC	13480	CAGCGCTGGA	13490	TGCCGGATA	13500	GGGGGGCAA
13520	GGGTTGCCGT	13530	TTTCATCATA	13540	CGTTGGGCTA	13550	TGGGGGGAAAAT	13560	13510	13580	AGCCGACAC
13590	GGGGATACTG	13600	ACGAAACGCC	13610	TTTAATCAGC	13620	CCAGTCCCAC	13630	GACGAAGCCG	13640	CCCTGTAAAC
13660	TTCGCAAAGG	13670	ATCAGCGGGC	13680	TGCCAGTATT	13690	GCCAAGAACCC	13700	TTACCCATCG	13710	13650

FIG. 10N

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13730	GCCGGAAAGG	13740	GCTGGTCTTC	13750	ATCCACGCGC	13760	GGCAAATAAT	13770	ATCGGGTGGCC	13780	GTGGGTGTCGG
13800	CTCCGGCGCC	13810	TTCATACTGC	13820	ACCGGGCGGG	13830	AGATTGAC	13840	CAGCGATACA	13850	GGCGGTCGTG
13870	ATTAGGCCG	13880	TGGCCTGATT	13890	CATTCCCCAG	13900	CGACCAGATG	13910	GGTGATTACG	13920	ATCGGGCTGC
13940	ACCATTGCGC	13950	TTACGGTTC	13960	GGTAGGCCAGC	13970	GGGGATCATC	13980	GGTCAGACGA	13990	TTCATTGGCA
14010	CCATGCCGTG	14020	GGTTCAATA	14030	GCTCATGCC	14040	GGGCCGTAG	14050	CGGTGGCAC	14060	GGGTGTACCA
14080	CAGCGGATGG	14090	TTCGGATAAT	14100	TTGGCTCAT	14110	CCACCACATA	14120	GCTTCATCAG	14130	14140
14150	TGCACCATCG	14160	TCTGCTCATC	14170	GGGAACAGCG	14180	AAGTTGTTCT	14190	GCTTCATCAG	14200	14210
14220	TGAGCAACGG	14230	CTTGGCGTTTC	14240	CATGACCTGA	14250	GATGATGCTC	14260	GTGACGGTTA	14270	ACGCCCTCGAA
14290	GGCTTCTGCT	14300	TCAATCAGCG	14310	AGCAGCAGCA	14320	AATCCGCACC	14330	14280	14290	CGACATCGCA
14360	ACAGTTTCGG	14370	TGCCGTGGC	14380	GGTGTGCAGT	14390	TCAACCACCG	14400	TGACATAGAG	14340	TCGGGGATT
14430	CATCGATAAT	14440	TTCACCGCCG	14450	TTTCAGACGTA	14460	GATGATGCTC	14470	14410	14420	CCACCAAGCT
14500	TGTTACCCGT	14510	AGGTAGTCAC	14520	TGCCGCTGGC	14530	GACCTGCGTT	14470	ATCGGCATAA	14480	14490
14570	TCATCATTAA	14640	CACGGAAAT	14650	AAAGGGCGGG	14600	GACCTGCGTT	14470	TGACATAGAG	14550	ATAAAGAAAC
					GCACATCTGA		ACTTCAGCCT		CCAGTACAGC		14560
					14590	14600	14540	14610	14620	14630	GGGGCTGAAA
					AACATGGAAA	14660	TCGCTGATT	14680	TTTATGCAGC	14690	AACGAGACGT
					CGCCACATAT	14670	CCTGATCTTC	14680	14700	14700	AGCGCAGCAC

FIG. 10P

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14710	CATCACCGCG	14720	AGGGGGTTT	14730	CTCCGGCGG	14740	TAAAATGCG	14750	CTCAGGTCAA	14760	14770
14780	TCCTGCCGT	14790	AACCGACCCAA	14800	GCGCCCCGTTG	14810	CACCACAGAT	14820	GAAACGCCGA	14830	14840
14850	TTGCCTCTG	14860	GCCTCCTGT	14870	AGCCAGGCTTT	14880	CATCAACATT	14890	AAATGTGAGC	14900	TCAAAAATAA
14920	CTCCGTGGGAA	14930	ACAAACGGCG	14940	GATTGACCGT	14950	AATGGGATAG	14960	GAGTAACAAC	14970	14980
14990	CGGTGATCT	15000	GCCAGTTGA	15010	GGGGACGGACG	15020	ACAGTATCGG	15030	GTCACGTTGG	15040	CGCATCGTAA
15060	CGGCACCGC	15070	TTCTGGTGCC	15080	GGAAACCAGG	15090	CCTCAGGAAG	15100	ATCGGACTCC	15110	15050
15130	AGGGGATCTG	15140	GTGCGGGCCT	15150	GCAAGCGCCA	15160	TTGCCATTTC	15170	AGGCTGGCA	15180	AGCCAGGCTT
15200	AGTTGGTAA	15210	GGCCAGGGTT	15220	CTTCGCTATT	15230	ACGCCAGCTG	15240	GATGTGCTGC	15250	15120
15270	GAAGCAGACG	15280	ACCTCCCGTT	15290	TTCCCAGTCA	15300	CGACGTTGTA	15310	AAACGACTTA	15320	AAGGGATTAA
15340	TAAACCAGAA	15350	CGGCAGCCAG	15360	GTGCAGCCAG	15370	CGGGGCCCTGC	15380	ATCCGTGAG	15390	GGGCTGCTC
15410	CCACCAAC	15420	CAAATTATAC	15430	GGGGGCCACC	15440	GCCGCCACCA	15450	GGCGACGGAA	15460	15330
15480	CTGCACCTT	15550	CACCAACATC	15560	GATGTCTGAA	15570	CTCCACCAAT	15580	AAAATCGCA	15590	GGAAACAAAC
15620	ACGGTCAAA	15630	ACATTGAGC	15640	AGACGACAGA	15650	TGGAAGCTAT	15660	TAGAAACGAA	15670	15680
					CCAAAAACGG		GTGAGGTGCA		CTCGTCTCAG		CCAGCCCCGC

FIG. 10Q

FIG. 10R

15690	CTGCCCTCC	15700	GATTATGGTA	15710	CAGCTCCCAT	15720	ACCGCCGCCA	15730	TTAGTAGATT	15740	TGCCGTCTGA	15750
15760	AATGTTACCA	15770	CCGCCTGCAC	15780	CATCGCTTTC	15790	TCTGAATTAA	15800	ATCGGGCAC	15810	AGTTAGATTG	15820
15830	AAACCGCCC	15840	GCAATCAGAA	15850	ATAATTCCAA	15860	AAGCTCAAC	15870	TACAAATTG	15880	ATCGGGACG	15890
15900	TGTTAGCCGA	15910	CACAAATTAAAT	15920	AGCGGTGTTG	15930	GGCTATGGC	15940	TCGGAAGCAA	15950	CTTCTAACGA	15960
15970	CGAGGGTTGG	16040	GACGACGACG	16050	ATAATCGGCC	16060	TAATAAGCT	16070	ATGTTAAATA	16080	TGTCCAAGCT	16090
16110	CGGGCAGCGT	16120	CCGCTTGGCA	16130	GAACATATCC	16140	ATCGCGTCCG	16150	CAGCCGCACG	16160	CGGCCATCT	16170
16180	GGGGGGTTGC	16250	TTTACTGGTT	16260	TGGGTCTGG	16270	GCATGATCGT	16280	GCTCCTGTCG	16290	GGCTAGGCTG	16300
16320	AAAACGTCTG	16330	CGACCTGAGC	16340	AAACAACATGA	16350	ATCACCATA	16360	TTGAGGCCAA	16370	CTGCTGTC	16380
16390	GGAAAGTCAGC	16400	GCCCTGCACC	16410	ATTATGTTCC	16420	GGATCTGCAT	16430	CGCAGGATGC	16440	CCTGTGGAAC	16450
16460	ACCTACATCT	16470	GTATTAACGA	16480	AGCGCTGGCA	16490	TTGACCCCTGA	16500	TCTGGTCCCG	16510	CGGCATCCAT	16520
16530	ACGCCAGTT	16540	GTTCACCCCTC	16550	ACAACGTTCC	16560	AGTAACCGGG	16570	ATCAGTAACC	16580	CGTATCGTGA	16590
16600	GCATCCTCTC	16610	TGTTTTCATC	16620	GGTATCATTAA	16630	CCCCCATGAA	16640	CAGAAATCCC	16650	AGGCATCAGT	16660
	GACCAAACAG		AAAAAACCG		CCCTTAACAT		GGCCCCGCTT		ATCAGAAGCC		GCTTCTGGAG	

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AAACTCAACG	16670	AGCTGGACGC	16680	GGATGAACAG	16690	GCAGACATCT	16700	GTGAATCGCT	16710	TCACGACCAC	16720	GCTGATGAGC
TTTACCGCAG	16740	CTGCCTCGCG	16750	CGTTTCGGTG	16760	ATGACGGGTGA	16770	AAACCTCTGA	16780	CACATGCAGC	16790	TCCCGGAGAC
GGTCACAGCT	16810	TGTCTGTAAG	16820	CGGATGCCGG	16830	GAGCAGACAA	16840	GCCC GTCA GG	16850	GCGCGTCAGC	16860	GGGTGTTGGC
GGGTGTCGGG	16880	GCGCAGCCAT	16890	GACCCAGTCA	16900	CGTAGCGATA	16910	GCGGAGTGTAA	16920	TACTGGCTTA	16930	ACTATGGGGC
ATCAGAGCAG	16950	ATTGTACTGA	16960	GAGTCACCA	16970	TATGGGGTGT	16980	GAAAATACCGC	16990	ACAGATGCCT	17000	AAGGAGAAAA
TACCGCATCA	17020	GGCGCTCTTC	17030	CGCTTCCTCG	17040	CTCACTGACT	17050	CGCTGCGCTC	17060	GGTCGTTCGG	17070	CTGCGGCGAG
CGGTATCAGC	17090	TCACTCAAAG	17100	GCGGTAATAAC	17110	GGTTATCCAC	17120	AGAATCAGGG	17130	GATAACGCCAG	17140	GAAAGAACAT
GTGAGCAAA	17160	GGCCAGCAA	17170	AGGCCAGGAA	17180	CCGTTAAAAG	17190	GCCGGTTGC	17200	GGCCGGTTTT	17210	CCATAGGCTC
CGCCCCCTG	17230	ACGAGGCATCA	17240	CAAAAATCGA	17250	CGCTCAAAGTC	17260	AGAGGTGGCG	17270	AAACCCGACA	17280	GGACTATAAA
GATACCAGGC	17300	GTTCACCCCT	17310	GGAGGCTCCC	17320	TCGTGGCTC	17330	TCCTGTTCCG	17340	ACCCCTGCCG	17350	TTACCGGATA
CCTGTCCGCC	17370	TTCTCCCTT	17380	CGGGAAAGCGT	17390	GGCGGCTTCT	17400	CATAGCTCAC	17410	GCTGTAGGTA	17420	TCTCAGTTCG
GTGTAGGTCTG	17440	TTCGCTCCAA	17450	GCTGGGCTGT	17460	GTGCACGAAC	17470	CCCCGTTCA	17480	GCCCGACCGC	17490	TGGCCTTAT
CGGGTAACTA	17510	TGTTCTTGAG	17520	TCAAACCCGG	17530	TAAGACACGA	17540	17550	17560	CTGGCAGCAG	17570	CCACTGGTAA
CAGGATTAGC	17580	AGAGCGAGGT	17590	ATGTTAGGCCG	17600	TGCTACAGAG	17610	CTTATCGCCA	17620	17630	17640	CTACGGCTAC

FIG. 10S

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17650	ACTAGAAGGA	17660	CAGTATTGG	17670	TATCTGGC	17680	CTGCTGAAGC	17690	CAGTTACCTT	17700	CGAAAAAGA	17710	GTTGGTAGCT
17720	CTTGATCCGG	17730	CAAACAAACC	17740	ACCGCTGGTA	17750	GGGGTGGTT	17760	TTTGTGTC	17770	AAGCAGCAGA	17780	TTACGGCGAG
17790	AAAAAAAGGA	17800	TCTCAAGAAG	17810	ATCCCTTGAT	17820	CTTTCTACG	17830	GGGTCTGACG	17840	CTCAGTGGAA	17850	CGAAAACCTCA
17860	CGTTAAGGGA	17870	TTTGGTCAT	17880	GAGATTATCA	17890	AAAAGGATCT	17900	TCACCTAGAT	17910	CCTTTAAAT	17920	TAAAATGAA
17930	GTTTAAATC	17940	AATCTAAAGT	17950	ATATATGAGT	17960	AAACTGGTC	17970	CAATGCTAAC	17980	TCAGTGGGC	17990	TCAGTGGGC
18000	ACCTATCTCA	18010	GCGATCTGTC	18020	TATTTCGTTT	18030	ATCCATAGTT	18040	CCGTGACTCC	18050	GATAACTACG	18060	GATAACTACG
18070	ATACGGGAGG	18080	GCTTACCATC	18090	TGGCCCCAGT	18100	GCTGCAATGA	18110	CCCACGCTCA	18120	CCGGCTCCAG	18130	CCGGCTCCAG
18140	ATTATCAGC	18150	AATAAACCAG	18160	CCAGCCCCAA	18170	GGGGCGAGGC	18180	18190	18200	TATCCGCCCTC	18210	TATCCGCCCTC
18210	CATCCAGTCT	18220	ATTAAATTGTT	18230	GCGGGGAAGC	18240	TAGAGTAAGT	18250	CCTGCAACTT	18260	TTAATAGTTT	18270	GGCACAACGTT
18280	GTGCCATTG	18360	CTGCAGGCAT	18300	CGTGGGTCA	18310	AGTTGCCAG	18320	18330	18340	TCCGGTTCCC	18350	TCCGGTTCCC
18350	AACGATCAAG	18430	GGGAGTTACA	18370	TGATCCCCA	18380	CGCTCGTCGT	18390	AGCTCCTCG	18400	GTCCTCCGAT	18410	GTCCTCCGAT
18420	CGTTGTCAGA	18440	AGTAAGTTGG	18440	TGGTGTGCAA	18450	AAAAGCGGTT	18460	18470	18480	TTCTCTTACT	18490	TTCTCTTACT
18490	GTCATGCCAT	18500	CCGTAAGATG	18510	ATCACTCATG	18520	GTTATGGCAG	18530	CACTGCATAA	18540	GTCAACCAA	18550	GTCAACCAA
18560	TGGGGGACCC	18570	GAGTTGCTCT	18580	CTTTTCTGTG	18590	ACTGGGTGAGT	18600	18610	18620	GAATAGTGTA	18630	GAATAGTGTA

FIG. 10T

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18630	AGTGCTCATC	18640	ATTGGAAAC	18650	GTTCTTCGGG	18660	GCGAAAACTC	18670	TCAAGGATCT	18680	GAGATCCAGT
18700	TCGATGTAAC	18710	CCACTCGTC	18720	ACCCAACGTGA	18730	TCTTCAGGCAT	18740	CTTTTACTTT	18750	TCTGGGTGAG
18770	AACAAACAGG	18780	AAGGCCAAAT	18790	GCCGCCAAAA	18800	AGGGATAAAG	18810	GGGACACGG	18820	TACTCATACT
18840	CTTCCTTTT	18850	CAATATTATT	18860	GAAGCATTAA	18870	TCAGGGTTAT	18880	TGTCCTCATGA	18890	GGGGATAACAT
18910	ATTTAGAAAA	18920	ATAAACAAT	18930	18940	18950	18960	18970	18980	18990	ATTTGAATGT
18980	CCATTATTAT	19050	CATGACATTA	19060	ACCTATAAAA	19070	CGCACATTTG	19080	CCCGAAAAGT	19090	GTCTAAGAAA
							CGCACATTTC		GCCACCTGAC		19040
							19010		19020		19030
							ATAGGGTAT		CACGAGGCC		AAGAA..
							19070		TTTCGTCTTC		

FIG. 10U

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/03935

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/00	C12N15/85	C12Q1/68	C12N5/10	C12N9/12
C12N15/13	C07K16/28	C12N15/12	C07K14/705	G01N33/53
C12N15/62	C07K19/00			

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C12Q C07K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 94 11523 A (IDEC PHARMACEUTICALS CORPORATION (US); REFF MITCHELL E. (US)) 26 May 1994 cited in the application see abstract see page 9, line 21 - page 10, line 29 see page 41, line 19 - page 42, line 19; figure 6 ---	1, 4-8, 11, 12, 25-29, 31, 32
A	US 5 464 764 A (CAPECCHI MARIO R. AND KIRK THOMAS R.) 7 November 1995 see abstract see column 13, line 32 - column 14, line 5 ---	1
A	WO 94 05784 A (UNITED STATES AMERICA REPRESENTED BY THE SECRETARY US DPT. AGRICULTURE) 17 March 1994 see abstract ---	1
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 Further documents are listed in the continuation of box C. Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
23 July 1998	05/08/1998
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Macchia, G

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International Application No

PCT/US 98/03935

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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